

PRENATAL PHTHALATE EXPOSURES AND CHILDHOOD ADIPOSITY:
EXAMINING THE OBESOGEN HYPOTHESIS

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ABSTRACT

Jessie P. Buckley: Prenatal phthalate exposures and childhood adiposity: examining the
obesogen hypothesis
(Under the direction of Stephanie M. Engel)

Background: Phthalate exposures, particularly during fetal development, are hypothesized to be obesogenic with effects of anti-androgenic phthalates potentially differing by child's sex. Previous studies are primarily cross-sectional and did not evaluate gestational exposures. To address these gaps, we assessed associations of prenatal phthalate exposures with childhood body size in three prospective birth cohorts.

Methods: In Aim 1, we utilized data from the Mount Sinai Children's Environmental Health Study (MSSM) to assess associations of third trimester maternal urinary phthalate metabolite concentrations with percent fat mass among children aged 4 to 9 years (N = 180 children with 364 visits). In Aim 2, we pooled data from three Children's Environmental Health Studies to examine prenatal maternal urinary phthalate concentrations in relation to overweight/obese status and age- and sex-standardized body mass index (BMI), weight, and height z-scores among children aged 4 to 7 years (N = 707 children with 1416 visits). For both studies, we estimated associations between standard deviation increases in natural log phthalate metabolite concentrations and longitudinal body size measures using Bayesian multilevel logistic and linear regression. We estimated associations in multiple metabolite models adjusted for confounders such as maternal pre-pregnancy BMI, gestational weight gain, smoking during pregnancy, and breastfeeding. We evaluated heterogeneity of associations by child's sex and, in the pooled study, by race/ethnicity and cohort.

Results: In Specific Aim 1, children in the highest tertile of creatinine-corrected maternal third trimester urinary concentrations of summed di-(2-ethylhexyl) phthalate metabolites (Σ DEHP) had lower percent fat mass than children in the lowest tertile ($\beta = -3.06$, 95% CI: -5.99, -0.09). In Specific Aim 2, prenatal urinary mono-3-carboxypropyl phthalate (MCP) concentrations were associated increased odds of overweight/obese status (OR per SD = 2.12, 95% CI = 1.16, 3.96). We also observed inverse associations of maternal urinary mono-ethyl (MEP) and mono-benzyl (MBzP) concentrations with z-scores among girls and non-Hispanic black children, respectively.

Conclusions: Our findings suggest that prenatal exposure to MCP, but not other phthalates, may be obesogenic. Associations of Σ DEHP, MEP, and MBzP with decreased body size in some subgroups indicate that prenatal exposures to these phthalates may also interfere with physical development.

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LIST OF ABBREVIATIONS AND SYMBOLS

BIA	Bioelectrical impedance analysis
BMI	Body mass index
BzBP	Benzylbutyl phthalate
CCEH	Columbia Center for Children's Environmental Health Study
CDC	Centers for Disease Control and Prevention
CI	Credible interval
DBP	Dibutyl phthalates
DEHP	Di-(2-ethylhexyl) phthalate
DEP	Diethyl phthalate
DIDP	Di-isodecyl phthalate
DINP	Di-isononyl phthalate
DMP	Dimethyl phthalate
DnOP	Di-n-octyl phthalate
EPA	Environmental Protection Agency
GED	General Educational Development
HOME	Health Outcomes and Measures of the Environment Study
IGF-1	Insulin-like growth factor 1
LOD	Limit of detection
MAR	Missing at random
MBzP	Mono-benzyl phthalate
MCMC	Markov chain Monte Carlo
MCNP	Mono-(carboxynonyl) phthalate
MCOP	Mono-(carboxyoctyl) phthalate
MCPP	Mono- (3-carboxypropyl) phthalate
MECPP	Mono- (2-ethyl-5-carboxypentyl) phthalate

MEHHP	Mono- (2-ethyl-5-hydroxyhexyl) phthalate
MEHP	Mono-2-ethylhexyl phthalate
MEOHP	Mono- (2-ethyl-5-oxohexyl) phthalate
MEP	Mono-ethyl phthalate
MiBP	Mono-isobutyl phthalate
MMP	Mono-methyl phthalate
MNAR	missing not at random
MnBP	Mono-n-butyl phthalate
MSSM	Mount Sinai Children's Environmental Health Study
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute for Environmental Health Sciences
NTP	National Toxicology Program
OR	Odds ratio
PPAR	Peroxisome proliferator-activated receptor
SD	Standard deviation
SES	Socioeconomic status
T4	Thyroxine
T3	Triiodothyronine
US	United States

CHAPTER I. INTRODUCTION AND SPECIFIC AIMS

A. Introduction

Childhood obesity rates increased substantially in the past several decades (Ogden and Carroll 2010). According to the most recent National Health and Nutrition Examination Survey (NHANES) population-based survey, 1 in 10 US children aged 2 to 5 years and nearly 1 in 5 children aged 6 to 19 years meets the definition of obese (Ogden et al. 2010). While energy balance is a key determinant of weight change, evidence suggests that well-known causes of obesity, including poor diet, physical inactivity, and genetic predisposition, may not fully account for the rapid increase in obesity rates (Heindel and vom Saal 2009; Keith et al. 2006).

Laboratory research supports the hypothesis that common environmental chemicals may negatively impact lipid regulation in favor of weight gain (Fromme et al. 2007; Heindel and vom Saal 2009; Janesick and Blumberg 2011). However, epidemiological studies of environmental causes of obesity are limited.

Human exposure to phthalates is ubiquitous and a number of lines of evidence point to phthalates as possible obesogens. Although there is currently no epidemiologic literature regarding prenatal exposure to phthalates and childhood growth, studies suggest that other endocrine disrupting chemicals may affect risk of overweight and obesity (Blanck et al. 2002; Gladen et al. 2000; Hertz-Picciotto et al. 2005; Karmaus et al. 2009; Lamb et al. 2006; Mendez et al. 2011; Smink et al. 2008; Verhulst et al. 2009). In addition, toxicologic research supports the hypothesis that early life phthalate exposures may affect fat accumulation (Feige et al. 2010; Hao et al. 2012; Schmidt et al. 2012) or pathways associated with offspring obesity (Bility et al. 2004; Desvergne et al. 2009; Feige et al. 2010; Hurst and Waxman 2003; Lampen et al. 2003;

Maloney and Waxman 1999; Sargis et al. 2009). Epidemiologic studies, primarily cross-sectional, have reported associations between phthalate metabolite concentrations and body mass index (BMI), obesity, weight, or waist circumference in children (Boas et al. 2010; de Cock et al. 2014; Hatch et al. 2008; Teitelbaum et al. 2012; Trasande et al. 2013; Wang et al. 2013). Furthermore, effects of phthalates on body size may differ by child's sex for metabolites of phthalates with anti-androgenic activity, as has been reported in studies of other developmental endpoints (Engel et al. 2009; Swan et al. 2010; Wolff et al. 2008). Identifying a link between prenatal phthalate exposures and childhood adiposity could have a substantial public health impact given that nearly 40% of US children are overweight or obese (Ogden et al. 2010).

The goal of this dissertation is to examine associations between phthalate concentrations measured in maternal urine collected during pregnancy and longitudinal measures of body size assessed during early childhood (ages 4 to 9 years) in three US birth cohorts: the Mount Sinai School of Medicine Children's Environmental Health Center (MSSM) Study, the Columbia Center for Children's Environmental Health (CCEH) Study, and Health Outcomes and Measures of the Environment (HOME) Study.

In Specific Aim 1, we examine the relationship between third trimester maternal urinary phthalate concentrations and percent fat mass among children aged 4 to 9 years in the MSSM cohort and evaluate effect measure modification by child's sex. Bio-electrical impedance analysis was used to measure body composition (including percent fat mass) at follow-up visits scheduled at approximately ages 4-5.5, 6, and 7-9 years. MSSM is the only cohort that collected body composition data, which provides quantitative estimates of fat mass and is a more sensitive indicator of increased adiposity than anthropometry alone. Associations between phthalate metabolite concentrations and BMI z-scores are also estimated with the goals of 1) assessing the degree to which associations between body composition and BMI z-scores agree (i.e., is BMI z-score sufficiently sensitive to detect associations between phthalate metabolite

concentrations and fat mass?) and 2) facilitating comparisons with other studies that do not have body composition data with which to evaluate potential obesogenic effects.

In Specific Aim 2, we estimate associations between phthalate concentrations measured in maternal urine collected during pregnancy and anthropometry outcomes (overweight or obesity, and weight, height, and BMI z-scores) collected at ages 4 to 7 years in the MSSM, CCEH, and HOME birth cohorts. All three cohorts collected longitudinal measures of anthropometry using similar standardized protocols. This aim builds upon the findings of Aim 1 by replicating analyses of associations between prenatal phthalate concentrations and anthropometry in two additional cohorts. Furthermore, pooling these data provides increased sample size to detect small effect sizes and explore effect measure modification by factors such as child's sex and race/ethnicity.

For both aims, associations between biomarkers of prenatal phthalate exposure and longitudinal outcome measures are estimated using mixed effects models to account for repeated measures for each child. We fit these models using a flexible Bayesian framework to simultaneously account for the issues of missing exposure (metabolite concentrations below the limits of detection), covariate, and outcome data. We adjust for important confounders including maternal pre-pregnancy BMI, gestational weight gain, smoking during pregnancy, and breastfeeding and estimate associations in multiple metabolite models to account for potential confounding among correlated metabolites.

B. Specific Aims

Specific Aim 1

Estimate associations of third trimester urinary phthalate metabolite concentrations with longitudinal assessments of percent fat mass collected from children aged 4 to 9 years in the MSSM cohort.

Sub Aim 1.1: Examine effect measure modification by child's sex.

Sub Aim 1.2: Estimate associations with BMI z-scores.

Specific Aim 2

Estimate associations of prenatal urinary phthalate metabolite concentrations with longitudinal assessments of overweight/obese status and BMI, weight, and height z-scores among children aged 4 to 7 years in the MSSM, CCEH, and HOME cohorts.

Sub Aim 2.1: Examine effect measure modification by cohort, child's sex, and race/ethnicity.

Hypotheses

We hypothesize that higher prenatal phthalate concentrations will be positively associated with body size outcomes related to body fatness (i.e., percent fat mass, BMI, weight, and overweight/obese status) measured among children. Metabolites of di-(2-ethylhexyl) phthalate (DEHP), dibutyl phthalates (DBP), and butylbenzyl phthalate (BzBP) will be most strongly associated with fat and body size outcomes. Associations will be stronger in boys, particularly for metabolites of phthalates with anti-androgenic activity (e.g., DEHP, DBP, and BzBP).

CHAPTER II. REVIEW OF THE LITERATURE

A. Childhood Obesity

Prevalence and Impact of Childhood Obesity

Childhood overweight and obesity, defined as greater than the 85th and 95th percentile of BMI for age and sex among children under age 20, is an emerging epidemic in the United States (US) and around the world. Globally, over 43 million children under the age of 5 are overweight, of whom 35 million live in developing countries (World Health Organization 2011). In the US, 11.9% of children aged 2-19 are obese and 31.7% are overweight (Ogden et al. 2010). Children of African American and Hispanic ethnicity have a particularly high prevalence of overweight and obesity. The prevalences of overweight and obesity are higher among both non-Hispanic black (35.9%, 20.0%) and Hispanic (38.2%, 20.9%) children aged 2-19 compared to non-Hispanic white children (29.3%, 15.3%) (Ogden et al. 2010).

Obese children are at risk of becoming obese adults (Serdula et al. 1993), with the concomitant risks of diabetes, heart disease, cancer, and many other conditions. In addition, overweight and obese children exhibit early physiologic changes associated with these chronic health conditions. For example, a recent systematic review and meta-analysis reported that obese children aged 5 to 15 have signs of early cardiovascular disease including higher systolic and diastolic blood pressure, cholesterol, triglycerides, fasting insulin, insulin resistance, and left ventricular mass compared to their normal-weight peers (Friedemann et al. 2012). The economic cost of childhood obesity is also substantial. The estimated annual cost of treating obesity-related diseases in children aged 6-17 increased from \$35 million in 1979-1981 to \$127 million in 1997-1999 (Wang and Dietz 2002).

Measuring Body Size in Children

This dissertation utilizes two methods of assessing child body size: 1) bio-electrical impedance analysis (BIA) of body composition, and 2) anthropometric measurements of body size.

BIA is a body composition measurement method that provides estimates of fat-free mass and fat mass. BIA requires that a person stand barefoot on a device resembling a scale to make contact with electrodes that send current through the body. BIA measures impedance, or opposition to a weak electrical current flowing through body tissues. Lean body mass has a low impedance because it is mostly aqueous and conducts electricity well, whereas fat mass has high impedance. Impedance is proportional to total body water and is used to estimate fat-free mass.

As BIA is an indirect method for assessing body composition, estimates of fat-free mass are obtained by using the measured impedance values in prediction equations developed against a reference standard. Based on a two-compartment model of the body (fat mass and fat-free mass), fat-free mass can be subtracted from the individual's weight to obtain an estimate of fat mass (Ellis 2001; Houtkooper et al. 1996). Percent body fat is then estimated as $100 \times (\text{weight} - \text{fat free mass}) / \text{weight}$ (Ellis 2001). Fat mass index and fat-free mass index are height-adjusted measures of fat and lean mass (fat mass index = $\text{fat mass} / \text{height}^2$, fat-free mass index = $\text{fat-free mass} / \text{height}^2$) (Freedman et al. 2005b; Lohman and Going 2006).

BIA may provide more precise estimates of adiposity than anthropometry since it distinguishes between fat and lean mass. There is known variation in fat mass within a given BMI and vice versa, particularly in children (Ellis 2001; Wright et al. 2008). For example, a high BMI can be achieved through greater muscle or bone mass rather than fat mass (Lohman and Going 2006). BIA provides a means for identifying children with excess fat versus children who are heavy due to greater lean mass, which is important in terms of future disease risk.

BIA relies on the assumption that fat-free mass has a relatively constant water content, which may not be the case among young children (Ellis 2001). Using pediatric-specific prediction equations helps to reduce misclassification of fat-free mass. Tanita leg-to-leg BIA does not perform as well as gold standard estimators of body density such as underwater weighting or total body water by isotope dilution. However, validation studies have reported good performance of BIA measurements in comparison to gold standard measures (Jebb et al. 2000; Nunez et al. 1997; Xie et al. 1999) and BIA is considered a valid estimator of adiposity for epidemiology studies (Ellis 2001; Houtkooper et al. 1996). Benefits of BIA in comparison to other body composition techniques are that BIA is low cost, portable, and non-invasive (Houtkooper et al. 1996).

In contrast to BIA, anthropometry is an inexpensive and non-invasive method for measuring body characteristics that is commonly used in epidemiology studies. It includes measures of height, weight, and circumferences or lengths of various body regions. Anthropometric measurements that are used to assess body fatness include metrics such as weight, BMI, or waist circumference. Because weight is a measure of total mass that incorporates muscle, bone, organs, and fat, greater weight does not necessarily indicate greater adiposity. BMI is a measure of excess weight for height [$\text{BMI (kg/m}^2\text{)} = \text{weight (kg)}/\text{height (m)}^2$] that is widely used in field studies as an indicator of adiposity. Waist circumference is a measure of central adiposity.

Raw anthropometric measurements can be used on their own for outcomes studies but they do not account for natural changes in growth patterns over childhood (Kuczmarski et al. 2002). Instead, raw measures are often converted to percentiles or z-scores in order to compare a child's weight, height, waist circumference, or BMI to a reference population. Percentiles and z-scores are usually age- and sex- specific and can be further customized (e.g., race or ethnic groups). Percentiles are used to determine the percent of a reference population that would have a value less than an individual's value. For example, a BMI percentile of 85 indicates that

85% of the reference population has a BMI percentile of less than 85. Overweight and obesity are clinical definitions of excess body fat that are defined using BMI percentiles, where overweight is greater than the 85th percentile and obesity is greater than the 95th percentile. Z-scores represent the difference between the individual's value and the mean value in the reference population divided by the standard deviation in the reference population. Z-scores are useful for longitudinal modeling of growth as the expected value at any time point is 0 if the population is a random sample of the population used to create the reference. Further, z-scores are standardized to the population standard deviation, reducing likelihood of heteroscedasticity. The 2000 CDC growth charts are often used in epidemiology studies to calculate age- and sex-specific percentiles or z-scores in reference to the US population (Kuczmarski et al. 2002). Using age- and sex-standardized measures facilitates valid comparisons in pediatric studies since children are in a period of rapid change with patterns that differ between girls and boys.

In children, BMI is a moderately sensitive and highly specific indicator of adiposity, and adolescents with a high BMI are at greater risk for adverse health outcomes in adulthood (Freedman and Sherry 2009). Evidence suggests that body composition methods may not outperform anthropometry when assessing associations between adiposity and metabolic risk factors (Stevens et al. 2008). For example, studies of blood pressure, blood glucose, high-density lipoprotein cholesterol, insulin, and triglycerides in adults have reported that anthropometric measures such as BMI are as or more predictive of these physiologic markers of adiposity than BIA or dual-energy X-ray absorptiometry (Hemmingsson et al. 2009; Lee et al. 2008; Willett et al. 2006). A further benefit of using anthropometric measures is that they are more easily understood by the public and facilitate risk communication (Stevens et al. 2008).

B. Environmental Causes of Obesity

Emerging evidence suggests that factors other than energy balance may contribute to the rapid rise in obesity rates (Heindel and vom Saal 2009; Keith et al. 2006) and several discoveries have led to a search for environmental causes of obesity. First, the concomitant increase in production of synthetic chemicals with rising obesity rates has implicated environmental exposures as potential risk factors for obesity (Baillie-Hamilton 2002), although this putative link suffers from the ecological fallacy. Second, prenatal factors such as fetal growth restriction and gestational tobacco smoke exposure have been demonstrated to affect obesity risk, indicating that *in utero* environmental exposures may program an obesogenic phenotype (Braun et al. 2010a; Heindel and vom Saal 2009; Oken et al. 2008). Third, adipose tissue is now recognized to be an active endocrine organ with chemical and hormonal signaling, which makes it a potential target for endocrine disrupting chemicals (Cooke and Naaz 2004; Newbold et al. 2009). Taken together, these findings provide a basis for exploring the role of endocrine disrupting chemicals in promoting obesity.

The “environmental obesogen hypothesis” theorizes that prenatal exposure to endocrine disruptors increases susceptibility to obesity through a fetal programming mechanism that may 1) alter control of adipose tissue development pathways, 2) increase the number of fat cells, 3) modify food intake and metabolism, and 4) affect insulin sensitivity and lipid metabolism (Diamanti-Kandarakis et al. 2009; Grun and Blumberg 2009; Heindel and vom Saal 2009).

There is emerging human evidence of an association between prenatal or early life exposure to environmental endocrine disruptors and body size in childhood, particularly persistent organic pollutants (for example: (Blanck et al. 2002; Gladen et al. 2000; Gladen et al. 2004; Halldorsson et al. 2012; Hertz-Picciotto et al. 2005; Jacobson et al. 1990; Karmaus et al. 2002; Lamb et al. 2006; Mendez et al. 2011; Patandin et al. 1998; Smink et al. 2008; Valvi et al. 2012; Verhulst et al. 2009)). These studies have reported positive associations between early life dichloro-diphenyldichloroethylene (DDE) and hexachlorobenzene (HCB) exposure and

childhood growth, and associations with polychlorinated biphenyls (PCBs) that depend on gender, timing, and dose (Tang-Peronard et al. 2011). Overall, these studies provide evidence that endocrine disrupting chemicals may alter childhood growth and lend support to the obesogen hypothesis.

C. Phthalate Exposures and Health Effects

Phthalates are synthetic diesters of phthalic acid used in a variety of industrial and consumer products. Phthalates are often grouped by molecular weight, with low molecular weight phthalates having fewer than 8 carbons in the alkyl chain and high molecular weight having 8 or more carbons. High molecular weight phthalates are added to plastics to make them flexible and durable. Low molecular weight phthalates are used as adhesives, detergents, and solvents. Sources of phthalate exposure in consumer products are varied and include polyvinyl chloride plastics, building materials, medical devices, pharmaceuticals, automotive components, toys, food packaging, cosmetics, fragrances, and pesticides (Schettler 2006). Over three million metric tons are produced annually (Bizzari et al. 2000).

Human Exposure to Phthalates

Human exposure to phthalates is ubiquitous, arising from contact with a wide range of consumer products. Phthalates may enter the body through ingestion, inhalation, dermal absorption, or intravenous injection. Biomonitoring of the phthalate metabolites has identified virtually universal exposure internationally and across all age ranges. At least 10 metabolites are commonly detected and three metabolites, mono-ethyl (MEP), mono-n-butyl (MBP), and mono-benzyl (MBzP), were present in more than 97% of urinary samples analyzed in the 1999-2000 NHANES survey (Silva et al. 2004).

Widespread phthalate exposure has prompted concern about potential adverse health effects, particularly with respect to fetal exposures since phthalate metabolites can cross the placenta (Mose et al. 2007). Due to these concerns, some phthalates have been regulated in consumer products in the US and abroad ([Anonymous] 2005a, b, 2008). The US federal regulations focus on reducing childhood exposures DEHP, DBP, and BzBP by regulating their presence in bath toys or other small plastic toys that can be mouthed by children. However, fetal exposure to phthalates may occur through *maternal* contact with phthalate-containing products, and, in some circumstances, banned phthalate diesters have merely been replaced by others that are both unstudied and unregulated.

Biomarkers of Phthalate Exposure

Biomarkers of phthalate exposure are a useful dosimeter because they incorporate exposure from multiple routes and sources. Phthalates can be measured in urine, serum, saliva, seminal fluid, breast milk, amniotic fluid, meconium, and placenta. However, urinary biomarkers of phthalate metabolites are the preferred method of measuring human exposure (Calafat and McKee 2006; Hogberg et al. 2008). Metabolite levels in urine are higher than in other media, can be more precisely measured, and are less intrusive to study subjects than collection of other biologic specimens. Measurement of phthalate metabolites rather than diesters also reduces the risk of contamination from phthalate-containing laboratory equipment, whereas the use of phthalate metabolite concentrations in blood or blood products as exposure biomarkers is questionable (Calafat et al. 2013).

Phthalates are quickly metabolized and excreted in urine, with elimination half-lives of less than 24 hours (<3 hours for phthalates and <12 hours for metabolites) (Koch et al. 2005). Diesters are metabolized to primary and secondary metabolites that are more bioavailable in the body. Phthalates and their metabolites, as well as concentrations measured in a US population-based sample, are described by molecular weight class in Table 1.

Studies examining repeated urine samples over periods of up to 3 months have reported intraclass correlations between 0.2 and 0.8, indicating that a single sample may be useful to project long term exposure to some phthalates but not others (Adibi et al. 2008; Fromme et al. 2007; Hauser et al. 2004; Marcus et al. 2010). Within person reproducibility of urinary phthalate metabolite levels measured on consecutive days ranges from 0.5 to 0.8, depending on the phthalate (Hoppin et al. 2002). Thus, there is substantial variability in individual exposures over time and repeat samples are optimal for classifying an individual's phthalate exposures. However, many sources of phthalate exposure are frequent and may result in steady-state levels (Calafat and McKee 2006; Hauser et al. 2004). The probability of correctly classifying "high exposure" over a 6-12 week period by using a single sample was reported in two studies to range between 0.50 and 0.74, depending on the metabolite (Adibi et al. 2008; Hauser et al. 2004). Urinary concentrations of phthalate metabolites in a single urine sample may be moderately predictive of DBP, DiBP, DEP, and BzBP exposures during pregnancy, with greater variability for DEHP (Adibi et al. 2008; Braun et al. 2012; Ferguson et al. 2014a).

Table 1. Phthalate metabolite classification and concentrations measured in the 2001-2002 National Health and Nutrition Examination Survey (NHANES)

Class	Phthalate	Abbreviation	Metabolite	Abbreviation	NHANES* median (µg/g)	
					Total Population	Pregnant Women
<i>Low Molecular Weight</i>						
	Dibutyl	DBP	Mono-isobutyl	MiBP	2.5	3.3
			Mono-n-butyl	MnBP	17.4	22.7
	Diethyl	DEP	Mono-ethyl	MEP	97.4	306.4
	Dimethyl	DMP	Mono-methyl	MMP	1.3	2.1
<i>High Molecular Weight</i>						
	Benzylbutyl	BzBP	Mono-benzyl	MBzP	9.7	15.7
	Di-2-ethylhexyl	DEHP	Mono-2-ethylhexyl	MEHP	3.9	10.4
			Mono- (2-ethyl-5-hydroxyhexyl)	MEHHP	16.6	22.8
			Mono- (2-ethyl-5-oxohexyl)	MEOHP	11.2	17.8
			Mono- (2-ethyl-5-carboxypentyl)	MECPP	27.0 [†]	n/a
	Di-n-octyl	DnOP	Mono- (3-carboxypropyl)	MCPP	2.5	3.5
	Di-isodecyl	DIDP	Mono-(carboxynonyl)	MCNP	4.5 [‡]	n/a
	Di-isononyl	DINP	Mono-(carboxyoctyl)	MCOP	2.5 [‡]	n/a

* Median creatinine-corrected concentration among the total population (Centers for Disease Control and Prevention) and pregnant women (Ye et al. 2009)

[†] MECPP concentration in the 2003-2004 NHANES (not measured in 2001-2002)

[‡] MCNP and MCOP concentration in the 2005-2006 NHANES (not measured in 2001-2002)

Phthalate Health Effects

Phthalate metabolite biomarker concentrations are among the highest of all endocrine disrupting chemicals (EDCs), and phthalates interfere with hormone homeostasis in animal, human, and *in vitro* studies (Committee on the Health Risks of Phthalates 2008; Shen et al. 2009). Experimental animal studies have reported adverse reproductive and developmental outcomes due to phthalate exposure and suggest that phthalates may have endocrine-disrupting properties (Lyche et al. 2009). A collection of reproductive endpoints observed in male animals following phthalate exposures, termed the phthalate syndrome, includes reduced sperm counts, histological changes in the testes, cryptorchidism, hypospadias, and reduced fertility (National Science Foundation 2008). Developmental outcomes observed in animals exposed to phthalates include reduced weight gain and survival of pups, male reproductive tract malformations, neural tube defects, and developmental delays (National Toxicology Program (NTP) 2003a, b, c, d, e, f, 2006).

There is a small but growing epidemiologic literature on potential adverse human health effects of phthalates. Human *in utero* exposure has been linked to altered gestational duration, reduced anogenital distance in boys, and impaired behavior and executive functioning skills (Adibi et al. 2009; Engel et al. 2010; Latini et al. 2003; Swan et al. 2005; Swan 2008; Whyatt et al. 2009; Wolff et al. 2008). Reproductive, respiratory, metabolic, and thyroid effects in children and adults have also been reported (Bornehag and Nanberg 2010; Duty et al. 2003; Hatch et al. 2008; Hauser et al. 2006; Huang et al. 2007; Stahlhut et al. 2007). A recent review of the state of the literature on phthalate exposures and children's health concluded that consistent evidence from well-designed studies support the existence of an association between early life DEHP and BzBP exposures and allergic diseases, with more limited evidence for associations of phthalate exposures with infant birth size, gestational length, physical development, neurodevelopment, and, in male infants, shorter anogenital distance (Braun et al. 2013).

D. Phthalates and Childhood Obesity

Proposed Biologic Mechanisms

Evidence suggests that phthalates may promote an obesogenic phenotype by altering peroxisome proliferator-activated receptors (PPARs), affecting hormone levels, or acting as anti-androgens (Figure 1). Phthalate metabolites have been shown to modify PPARs alpha and gamma in animal models, which may influence developmental programming of the metabolic system by increasing adipogenesis and altering insulin and leptin levels. Animal evidence indicates that phthalate exposures affect hormone signaling and cross-sectional studies in humans have reported associations between concentrations of some phthalate metabolites and thyroid hormone levels. Additionally, several phthalates exhibit anti-androgenic activity, which may cause sex-specific changes in muscle and fat development.

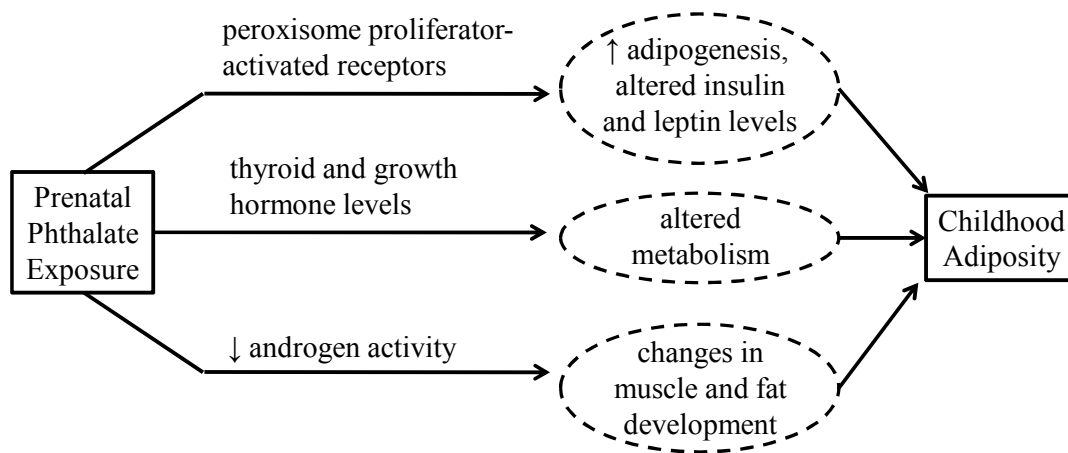


Figure 1. Association between prenatal phthalate exposure and childhood adiposity

PPARs are nuclear receptor proteins that activate gene expression and have important roles in regulating energy metabolism, cell proliferation, and inflammation. PPAR alpha is expressed in several body compartments including liver and adipose tissue and is an important regulator of glucose metabolism. Studies of PPAR alpha null mice have shown altered lipid

homeostasis and fatty acid metabolism. PPAR gamma is expressed in adipose tissue and plays a key role in adipogenesis, insulin sensitivity, and lipid storage.

Phthalate metabolites have been shown to modify PPARs alpha and gamma in animal models, which may influence developmental programming of the metabolic system by increasing adipogenesis and altering insulin and leptin levels (Bility et al. 2004; Boberg et al. 2008; Casals-Casas et al. 2008; Desvergne et al. 2009; Feige et al. 2010; Hurst and Waxman 2003; Lampen et al. 2003; Maloney and Waxman 1999; Sargis et al. 2009). The experimental animal literature has focused primarily on DEHP or its metabolites as they are known to be hepatotoxic and PPAR alpha is expressed in liver. These studies indicate that DEHP and its metabolites affect both PPAR alpha and gamma expression. Studies have also reported changes in PPAR alpha and gamma expression associated with DBP and BzBP but not DMP or DEP metabolites.

If phthalates affect obesity through a PPAR mechanism, associations might also be observed between phthalate exposures and diabetes because PPARs regulate glucose homeostasis. Cross-sectional studies have reported associations between phthalates and diabetes or insulin resistance in adults, though results were inconsistent regarding which phthalates were associated with outcomes and whether metabolites were associated with increased, decreased, or no difference in prevalence of diabetes (James-Todd et al. 2012; Lind et al. 2012b; Stahlhut et al. 2007; Svensson et al. 2011).

Thyroid and thyroid stimulating hormones are integral for regulating growth and metabolism. Experimental evidence indicates that phthalates affect thyroid hormone signaling (Breous et al. 2005; Ishihara et al. 2003; Shimada and Yamauchi 2004; Sugiyama et al. 2005; Wenzel et al. 2005). In rats, DEHP lowers free thyroxine (T4) levels and alters iodide uptake in thyroid follicular cells (Hinton et al. 1986; Howarth et al. 2001; Poon et al. 1997; Price et al. 1988; Wenzel et al. 2005) and DBP exposure causes reduced triiodothyronine (T3) and T4 levels (O'Connor et al. 2002). The growth hormone insulin-like growth factor 1 (IGF-1) may also

be influenced by phthalates. *In utero* DBP and DEHP exposure in rats has been reported to increase mRNA expression for IGF pathway genes (Bowman et al. 2005; Lin et al. 2008). Though cross-sectional studies do not provide causal evidence, reports of associations between urinary phthalate metabolites and thyroid hormones, including T3, T4, thyroglobulin, and thyroid-stimulating hormone, have generally found inverse associations (Boas et al. 2010; Huang et al. 2007; Meeker et al. 2007; Meeker and Ferguson 2011).

Androgens are sex steroid hormones with key roles in male reproductive development. Studies have consistently reported effects of *in utero* DEHP, BzBP, and DBP exposures on male androgen-related reproductive endpoints in rats and mice, including hypospadias, cryptorchidism, reduced testosterone production, and decreased sperm counts (Fisher 2004; Gray et al. 2006; Lottrup et al. 2006; Pan et al. 2006). Two prospective studies have examined early life phthalate exposures in relationship to androgen-mediated effects. Swan et al. reported associations between prenatal levels of MEP, MBP, and DEHP metabolites and shortened anogenital distance, reduced penile width, and incomplete testicular descent (Swan 2008). Main et al. reported correlations between MEP, MBP, MMP, MEHP, and MINP in breast milk and reproductive hormone levels in the serum of 3 month old boys (Main et al. 2006).

Androgens may also be linked to changes in adiposity since these hormones affect body composition by promoting skeletal muscle development and inhibiting lipid storage in fat cells. Since men have higher levels of androgens such as testosterone, they typically have more muscle and less fat than women. Androgen levels are associated with obesity and metabolic syndrome in adults, with notable differences in relationships by sex (Barber et al. 2006; Moulana et al. 2011). Low testosterone levels are associated with poorer cardiovascular health and larger waist circumference in men. In women, high testosterone levels are associated with increased BMI, metabolic syndrome, and polycystic ovary disease. Because phthalates inhibit androgen activity, it is hypothesized that effects of phthalates through this pathway would result in increased adiposity in men but decreased or no change in women.

Toxicological Evidence

Toxicological studies have directly assessed the role of early life exposure to DEHP or its hydrolytic metabolite, MEHP, in the development of obesogenic phenotypes, with results indicating species- and dose-specific effects. Two studies of perinatal MEHP or DEHP exposure in mice reported increased body weight and fat deposition in offspring, though one study observed effects only at the lowest dose (0.05 but not 0.25 or 0.5 mg MEHP/kg/day) (Hao et al. 2012) and the other reported effects only at the highest dose (5 but not 0.05 mg DEHP/kg/day) (Schmidt et al. 2012). Two studies of pregnant rats exposed to doses ranging from 1 to 400 mg DEHP/kg/day reported no difference in offspring total body weight (Campioli et al. 2014; Kobayashi et al. 2006). In contrast, relatively high dose DEHP exposure (2% by body weight) induced PPAR alpha-mediated reductions in body weight and fat mass in both rats (Itsuki-Yoneda et al. 2007) and mice (Xie et al. 2002). Interestingly, Feige et al. reported that although high dose DEHP exposure (500 mg/kg body mass/day) protected against diet-induced obesity via PPAR alpha activation in wild-type mice, mice with humanized PPAR alpha gained more weight and adipose tissue than untreated controls (Feige et al. 2010). While the causes of species differences remain unclear, effects of DEHP on other, less understood pathways (e.g., liver metabolism, thyroid function, or androgen activity) may play a role.

Epidemiological Evidence

Several studies, primarily cross-sectional, have reported associations between phthalate metabolite concentrations and BMI, obesity, weight, or waist circumference in children (Boas et al. 2010; de Cock et al. 2014; Hatch et al. 2008; Teitelbaum et al. 2012; Trasande et al. 2013; Wang et al. 2013) and adults (Hatch et al. 2008; Lind et al. 2012a; Song et al. 2014; Stahlhut et al. 2007). However, none assessed exposure to phthalates during gestation, which is thought to be a relevant critical time period for effects related to development of obesity (Dietz 1994). A

recent review of the literature on phthalates and body size concluded that findings are inconsistent regarding which phthalates are associated with outcomes or whether associations are positive or inverse (Goodman et al. 2014). Furthermore, a causal link between phthalate exposures and subsequent body size cannot be established in cross-sectional studies that measure concurrent exposures and outcomes (Engel and Wolff 2013).

In the only previous study of early life phthalate exposure and BMI in humans, de Cock et al. examined quartiles of DEHP metabolite concentrations measured in cord blood (likely reflecting primarily intrapartum exposures) in relation to repeated BMI measurements in the first year of life among 89 Dutch infants (de Cock et al. 2014). Children in the lowest MECPP quartile and boys in the lowest MEOHP quartile had higher BMI in the first year, though there was no evidence of dose-response and the same pattern was not observed for MEHHP, another secondary oxidative metabolite of DEHP. This study has several important limitations. Analyses were stratified by phthalate quartile and sex yielding very small sample sizes at each time point (<10). Further, the use of phthalate metabolite concentrations in blood or blood products as exposure biomarkers is questionable (Calafat et al. 2013). While the authors only measured secondary or oxidative metabolites, that may reflect true biological exposures, it is possible that cord plasma DEHP metabolites arise from hospital-based exposures to DEHP at the time of delivery (Vandentorren et al. 2011; Yan et al. 2009).

E. Knowledge Gaps

While there is evidence that prenatal exposure to other environmental EDCs affect childhood growth and may have sex-specific effects (Blanck et al. 2002; Gladen et al. 2000; Gladen et al. 2004; Hertz-Picciotto et al. 2005; Karmaus et al. 2009; Lamb et al. 2006; Mendez et al. 2011; Smink et al. 2008; Verhulst et al. 2009), no studies have yet prospectively examined prenatal phthalate exposure and body size in humans.

Cross-sectional analyses cannot disentangle the temporal ordering of exposure and outcome; it is unclear whether phthalate exposures are causally related to obesity or phthalate body burden differs in obese individuals due to differences in exposure to phthalate sources. For example, it is plausible that phthalate exposures differ among obese individuals due to differences in exposure through 1) dietary sources, both amount and types of foods (Serrano et al. 2014), 2) personal care product use, due to larger surface area, (Buckley et al. 2012; Duty et al. 2005; Just et al. 2010; Watkins et al. 2014) or 3) medications to treat comorbid conditions, since DBP and DEP are used to coat some medications (Hernandez-Diaz et al. 2013).

Previous studies have been unable to examine *prenatal* exposure, which is thought to be the relevant critical window for an effect of phthalates on adiposity. No prior studies of children have measured adiposity using bio-electrical impedance analysis, which is a strength of the current study. Finally, prior investigations of phthalates and of other EDCs have been limited in their ability to examine effect heterogeneity by sex due to small sample sizes or populations comprised of only males or females. This prospective study of prenatal phthalate exposures addresses the above gaps in the scientific evidence regarding the role of phthalates in affecting body size or promoting obesity in children.

CHAPTER III. METHODS

A. Study Design Overview

This dissertation assesses associations between maternal urinary phthalate metabolite concentrations during pregnancy and longitudinal measures of childhood body size among members of the MSSM, CCEH, and HOME birth cohorts. Specific Aim 1 assesses the association of third trimester maternal urinary phthalate concentrations and percent fat mass during childhood in the MSSM cohort, the only study that assessed body composition. Specific Aim 2 examines prenatal phthalate exposures in relation to anthropometry outcomes in all three cohorts and assesses effect measure modification and dose-response in this larger pooled sample. For both aims, we used a Bayesian multilevel modeling framework to incorporate multiple observations per child, stabilize estimates of correlated phthalate metabolites, and account for missing data in exposures, covariates, and outcomes.

B. Study Populations

The NIEHS/EPA funded Mount Sinai Children's Environmental Health and Disease Prevention Research Center enrolled 479 primiparous women from the Mount Sinai prenatal clinic and two adjacent private practices between 1998 and 2002. Women had to have had a prenatal care visit by 26 weeks gestation, no serious medical complications (hypertension, diabetes, thyroid disease), consumed less than 3 alcoholic beverages per day, and had no illegal drug use. Seventy-five women were subsequently excluded because of medical complications, very premature births (<32 weeks gestation or <1500 grams), delivery of an infant with birth defects, inability to obtain biological specimens before delivery, change of residence, or refusal to continue participation. The final cohort consists of 404 mother-infant

pairs for whom birth data are available. Children attended follow-up clinic visits scheduled at approximately ages 1, 2, 4, 6, and 7 years. Data regarding maternal characteristics were collected by an in person, two hour structured interview administered to mothers by trained research assistants during third trimester prenatal care visits. This questionnaire was administered to all mothers (n = 404) and ascertained information on environmental exposures, sociodemographic characteristics, maternal health, and lifestyle habits. Pregnancy and delivery characteristics, birth outcomes, gestational age, and infant sex were ascertained from the computerized perinatal database at Mount Sinai Hospital. Duration of breastfeeding was ascertained by maternal questionnaire at the earliest follow-up visit. A number of studies within this sample have been published (Berkowitz et al. 2003; Berkowitz et al. 2004; Engel et al. 2007; Engel et al. 2009; Engel et al. 2010; Wolff et al. 2007; Wolff et al. 2008).

The Columbia Center for Children's Environmental Health enrolled 727 pregnant women between 1998 and 2006. The cohort was restricted to non-smoking women 18-35 years old who self-identified as either African American or Dominican and who had resided in Northern Manhattan or the South Bronx in New York City for >1 year prior to pregnancy. Women were excluded if they used illicit drugs; had diabetes, hypertension, or known human immunodeficiency virus; or had their first prenatal visit after gestational week 20. Women were enrolled if a 48-hour personal air sample was collected during their third trimester and a blood sample was collected from the mother and/or newborn at delivery. The cohort was predominantly full term (no infant was born <32 weeks gestation or <1500g). Follow-up visits were conducted at 6 months and 1, 2, 3, 5, and 7 years. Investigations of this sample have been previously published (Adibi et al. 2008; Adibi et al. 2009; Just et al. 2010; Perera et al. 2006; Perera et al. 2003; Whyatt et al. 2009).

The Health Outcomes and Measures of the Environment (HOME) Study is an ongoing NIEHS/EPA funded prospective birth cohort. Among 1,263 eligible women, 468 enrolled in the HOME Study between March 2003 and January 2006. Because the HOME Study contains a

nested, randomized trial of in-home lead and injury hazard controls women had to be living in housing built before 1978. Additional eligibility criteria included: ≤ 19 weeks gestation upon enrollment; living in Brown, Butler, Clermont, Hamilton or Warren counties in Ohio; intention to continue prenatal care and deliver at collaborating obstetric practices; human immunodeficiency virus negative; and not receiving seizure, thyroid, or chemotherapy/ radiation medications. A total of 389 women delivered live-born, singleton infants without birth defects. Children returned for annual follow-up visits between 1 and 5 years of age. Characteristics of this sample have been previously described (Braun et al. 2009; Braun et al. 2010a; Braun et al. 2010b; Braun et al. 2011).

Human Subjects

Women provided informed consent prior to participation and children aged ≥ 7 years provided assent. The MSSM, CCEH, and HOME studies received approval from the Institutional Review Boards of the Mount Sinai School of Medicine, Columbia University, and the University of Cincinnati College of Medicine, respectively. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research.

This dissertation utilizes previously collected, de-identified data and no study participants were contacted for additional information. The project was approved by the Institutional Review Board of the University of North Carolina at Chapel Hill on February 13, 2012.

Study Sample for Specific Aim 1

Children in the MSSM study (N = 404) were invited to return for three follow-up visits scheduled at approximately ages 4-5.5 (mean=4.9), 6 (mean=6.2), and 7-9 (mean=7.8) years. Of the 188 children who returned for at least one follow up visit between ages 4 and 9 years,

maternal prenatal phthalate metabolite concentrations were available for 182 children. Following previous work (Wolff et al. 2008), we excluded two observations obtained from a very dilute urine sample (<10 mg/dL creatinine) due to the potential for inaccurate biomarker measurements. Thus, the final sample for Specific Aim 1 is 180 children with body composition and anthropometry measurements collected at a total of 364 follow up visits.

Study Sample for Specific Aim 2

Specific Aim 2 utilizes data from all three cohorts. These studies were designed with many common features and the feasibility of pooling data is supported by the following circumstances: (1) urine samples were collected during the same period of pregnancy and levels are comparable across cohorts; (2) urine samples were analyzed for phthalate metabolites at the same laboratory; (3) body size assessment protocols were performed in the same manner at each study site; (4) there are overlapping ages at which outcomes were assessed between the studies; and (5) there is information on key confounding factors available from all cohorts.

The study population for Specific Aim 2 includes children in the MSSM, CCEH, and HOME studies with measured maternal urinary phthalate concentrations during pregnancy. We excluded infants born at <32 weeks gestation or <1500 grams ($n = 1$ CCEH and 5 HOME cohort members) and samples with a very dilute urine concentration (<10 mg/dL creatinine, $n = 9$) (Wolff et al. 2008). Of the 1143 children meeting our baseline inclusion criteria, the final study sample included 707 children with weight and height data collected at one or more follow-up visits occurring between 4 and 7 years of age ($n = 1416$ follow-up visits).

C. Exposure Assessment

Data Collection

Women provided a spot urine sample at mean \pm standard deviation (SD) gestational ages of 31.6 ± 5.1 (MSSM), 34.4 ± 3.0 (CCEH), and 27.1 ± 2.2 (HOME) weeks. All three cohorts shipped third trimester spot urine specimens to the laboratory of Dr. Antonia Calafat at the Centers for Disease Control and Prevention (CDC) where samples were analyzed for phthalate metabolites. Sample collection and storage as well as analytic methods and quality control procedures have been previously described (Kato et al. 2005; Silva et al. 2008; Valentin-Blasini et al. 2007). The CDC lab is Clinical Laboratory Improvement Amendments certified and follows strict quality control protocols that are enforced by post-analysis independent statistical review before release of results. Internal batches include blanks and urine pools whose results must conform to a stipulated quality control range, or else the batch is rerun (Norrgran et al. 2006; Westgard et al. 1981). In addition, a blind pool is incorporated every tenth sample. When results for a batch's external quality control deviate from the overall values, CDC reruns that batch (specimens are retained until the study is complete). Correction factors were applied to MBzP (0.72) and MEP (0.66) concentrations and limits of detection (LOD) to adjust for inaccuracies in analytical standards (Centers for Disease Control and Prevention 2012).

Measures

Phthalate metabolites measured in all three cohorts include MBP, MIBP, MEP, MBZP, MCPP, MEHP, MEHHP, MEOHP, and MECPP. Following previous work (Wolff et al. 2008), we examined DEHP metabolites (MECPP, MEHHP, MEHP, MEOHP) as a micromolar sum (Σ DEHP, micromoles per liter) as they are highly correlated, represent exposure from the same sources, and cannot be independently intervened upon.

To facilitate comparison of phthalate effect sizes in each Aim, we standardized the natural log concentration of each phthalate metabolite to its mean and SD. For Specific Aim 2, we standardized metabolites to their distributions in the pooled sample. To examine non-monotonic associations (e.g., threshold effects), we also explored the form of exposure-response relationships using quantile groups (tertiles for Aim 1 and quartiles for Aim 2). For these analyses, we categorized exposure using creatinine-corrected concentrations (micrograms per gram creatinine for metabolites or micromoles per gram creatinine for Σ DEHP).

D. Outcome Assessment

Body Composition

MSSM assessed body composition of children using leg-to-leg BIA at study visits scheduled at approximately 4-5.5, 6, and 7-9 years of age (1-3 study visits per child). The Tanita scales were donated by the company and are not commercially available (Tanita TBF-300). These scales were specifically designed for use in pediatric research with features such as small foot pads. Study staff were trained to use the BIA equipment using standard protocols.

There is no agreed upon set of prediction equations for BIA analysis in children. The Tanita scales feature a built-in, proprietary pediatric prediction equation for calculating body composition estimates. The product specifications note that the built-in pediatric equations are valid for children aged 7 years and older. The prediction equations cannot be directly validated since body composition measures using gold or alloyed gold standard methods are unavailable in this cohort (e.g., dual-energy X-ray absorptiometry, underwater weighing).

As an exploratory analysis, we compared the body composition values calculated by the Tanita scale to values estimated using two validated equations in the published literature (Clasey et al. 2011a; Horlick et al. 2002). Clasey et al. created and cross-validated BIA body

composition equations in a population of 436 white, non-Hispanic children aged 5-11 (Clasey et al. 2011a). The study population included overweight and obese children in order to generate equations that are valid in these children, which have been underrepresented in samples used to validate other pediatric body composition equations. Unpublished work by these authors reported that the equation performs equally well among a sample of non-Hispanic black and bi-racial children (Clasey et al. 2011b). The Clasey et al. equation predicts fat-free mass on the basis of height, weight, and impedance values.

Horlick et al. generated equations based on a multi-ethnic population of 1170 New York City children aged 4-18 (Horlick et al. 2002). They published two sets of equations. The first equation predicts fat-free mass based on height, impedance, weight, age, and sex. The second equation additionally includes race/ethnicity and Tanner stage. Because Tanner staging is not available in the MSSM cohort, only the first equation was evaluated.

The Horlick et al. equation performed very poorly in our sample, with 103 of 364 fat-free mass estimates exceeding the child's total mass. Therefore, we did not evaluate this equation further. The Clasey et al. equation performed better, with estimated fat-free mass values exceeding total mass for only two children. The Pearson correlation coefficient between percent fat mass estimated using the Clasey et al. equation versus the built-in Tanita equation was very high (0.97).

This similarity is not unexpected, as previous studies have shown that various prediction equations perform similarly well in terms of 1) the correlation between predicted and observed total body water and fat-free mass (Horlick et al. 2002) and 2) the rank order of children by body fat mass (Williams et al. 2007). Based on this exploratory analysis, we used the Tanita proprietary equations as they did not have implausible estimates and produced fat mass values that are highly correlated with those estimated by an equation validated for the age range in the current study.

Anthropometry

Weight and height were measured at follow-up visits scheduled for approximately ages 4-5.5, 6, and 7-9 years (MSSM), 5 and 7 years (CCEH), and 4, 5, and 7-9 years (HOME). In all three cohorts, study staff obtained anthropometric measurements using standard, uniform protocols for standing height and weight. We obtained body size measures from children in bare or stocking feet wearing light clothing (i.e., pediatric gown, underwear, or shorts and a t-shirt). We measured weight using a digital scale (HOME and CCEH 5 year visit) or a pediatric Tanita scale (MSSM and CCEH 7 year visit) (models TBF-300 and BC-418, Tanita Corporation of America, Arlington Heights, Illinois) and determined height using wall-mounted stadiometers.

We calculated BMI as weight (kg) / height (m)² and computed age- and sex-standardized BMI, weight, and height z-scores and percentiles using a CDC SAS macro (Centers for Disease Control and Prevention 2004). For Specific Aim 2, we also classified children at each follow-up visit as overweight or obese if their age- and sex-standardized BMI percentile exceeded 85.

E. Covariates

Variables Included in Outcome Models

Each birth cohort administered questionnaires during pregnancy and early childhood and gathered additional information on the health status of mothers and children from medical and birth records. We identified variables on confounding pathways using directed acyclic graphs. These covariates included the following variables, which are described in greater detail below.

- Maternal race/ethnicity
- Maternal age
- Maternal education
- Maternal work status during pregnancy
- Parity (Specific Aim 2)

- Maternal smoking during pregnancy
- Maternal pre-pregnancy BMI
- Maternal height
- Adequacy of gestational weight gain (Specific Aim 1)
- Gestational weight gain (Specific Aim 2)
- Calendar date of urine collection
- Urine dilution
- Child's sex
- Breastfeeding
- Months of age at follow-up
- Physical activity (Specific Aim 1)
- Cohort (Specific Aim 2)

There are differences in both phthalate exposure patterns (Silva et al. 2004) and childhood obesity risk (Ogden et al. 2010) by race and ethnic group. We thus included race/ethnicity as a covariate and examined whether associations of phthalate metabolites with childhood body size were heterogeneous by race/ethnicity. Mother's self-reported race/ethnicity was categorized as non-Hispanic white, non-Hispanic black, Hispanic, or other. In Specific Aim 1, we grouped children of Hispanic and other race/ethnicity based on the similarity of their outcome and covariate distributions. In Specific Aim 2, all four categories were included.

Phthalate exposures vary by age (Silva et al. 2004) and maternal age is a known risk factor for various pregnancy outcomes including fetal growth. Maternal age at delivery was ascertained using the mother's birth date and the date of delivery in all three cohorts.

Measures of socioeconomic status (SES) are associated with phthalate levels in women of reproductive age. For example, lower socioeconomic status is associated with higher levels

of MBZP and lower levels of DEHP metabolites (Kobrosly et al. 2012). Prevalence of childhood obesity also varies by SES. Therefore, we included two measures of maternal SES in our analyses: maternal education and maternal work status during pregnancy. Maternal education was ascertained using the baseline questionnaire in all cohorts. We categorized education as <high school, high school or General Educational Development (GED), some college, or ≥college degree. For Specific Aim 1, we further dichotomized maternal education as <college degree or ≥college degree based on distributions with exposures and outcomes. For Specific Aim 2, we included all four categories. Maternal work status was ascertained by questionnaire. For both Aims, we classified women as either employed or as a homemaker or student.

Parity is associated with birth weight, which is in turn related to childhood adiposity, and increasing number of siblings has been associated with lower BMI. Parity may also be related to differences in phthalate exposure sources such as personal care product use. Specific Aim 1 did not include this variable as MSSM restricted to primiparous women. For Specific Aim 2, we assessed parity by questionnaire at baseline and classified women as primaras or multiparas. We also examined modification by parity as a sensitivity analysis exploring the influence of cohort restriction criteria.

Gestational smoke exposure is a risk factor for childhood obesity (Oken et al. 2008). We classified pregnancy smoking status as either active or passive using different criteria for each cohort. For MSSM, we determined maternal smoking during pregnancy based on self-report. For HOME, we classified women as active smokers during pregnancy if the average of three cotinine concentrations (measured at 16 weeks, 26 week, and birth) exceeded 3 ng/mL (Benowitz et al. 2009). CCEH excluded women with evidence of active smoking based on either self-report or cotinine concentrations. As we did for parity, we also examined modification by maternal smoking during pregnancy as a sensitivity analysis for cohort restriction criteria in Specific Aim 2.

Maternal BMI is a strong predictor of offspring BMI (Salsberry and Reagan 2005; Weng et al. 2012) and may be associated with differences in phthalate exposure sources (e.g., diet, personal care products, medication use). We calculated pre-pregnancy BMI based on maternal self-reported weight and height (kg/m^2) at enrollment. We adjusted for maternal height due to its genetic influence on child body size.

Gestational weight gain may be associated with offspring adiposity (Weng et al. 2012) and with phthalate exposures through diet, personal care products, or medication use. We calculated gestational weight gain as the last pregnancy weight (measured or reported by the mother) minus self-reported pre-pregnancy weight. For Specific Aim 1, we calculated adequacy of gestational weight gain as the ratio of observed gestational weight gain to the expected gestational weight gain based on the 2009 Institute of Medicine recommendations: $(\text{observed weight gain} / \text{expected weight gain}) \times 100$ (Bodnar et al. 2010). Expected weight gain was calculated as follows: recommended weight gain in the first trimester + (gestational age at delivery – 13 weeks) \times recommended second and third trimester weekly rate of gain. Recommended weight gains are based on the mother's pre-pregnancy BMI. We used the continuous adequacy of weight gain variable in all models. For tabular display of distributions, adequacy of weight gain was categorized as less than recommended ($<86\%$), recommended ($86\text{--}120\%$), or more than recommended ($>120\%$) (Bodnar et al. 2011). We did not calculate adequacy of gestational weight gain for Specific Aim 2 as pre-pregnancy BMI was missing for some CCEH and HOME participants and therefore we were not able to determine recommended weight gains for each trimester of pregnancy. Instead, we adjusted for gestational weight gain (lbs) as a continuous variable.

We adjusted for calendar date of urine collection to account for temporal trends in phthalate exposures and prevalence of childhood obesity.

Creatinine and specific gravity are used in studies of urinary exposure biomarkers to correct for urine dilution. MSSM and HOME measured creatinine in maternal urine samples.

CCEH measured specific gravity in all urine samples ($n = 339$), with creatinine additionally measured in a subset ($n = 137$). Methods for adjusting for urine dilution in Specific Aim 2 are described below.

Child's sex was ascertained from birth records and included as a covariate in both Specific Aims. There are sex differences in the distribution, amount, and timing of fat accumulation in boys and girls. Because there may be anti-androgen mediated effects of phthalate exposures on body size, child's sex was evaluated as an effect measure modifier.

Although breastfeeding occurs after phthalate exposure, it has been associated with childhood BMI (Weng et al. 2012) and may lie on an unblocked backdoor path between phthalate exposures and body size outcomes. Breastfeeding was assessed by questionnaire at follow-up and categorized as ever/never.

We adjusted for age at follow-up (in months) to account for variation in child body size measures by age. In models of continuous outcomes, we also included an interaction between age at follow-up and child's sex due to differences in distributions by age and sex.

In an effort to increase precision of our estimates for Specific Aim 1, we adjusted for physical activity at each follow-up visit. For MSSM, we classified children as active if the parent/caretaker reported the child was "active most of the time" or inactive if the child was "active some of the time" or "hardly at all". Physical activity information was not available for CCEH or HOME and was therefore not considered for inclusion in Specific Aim 2.

Finally, for Specific Aim 2, we adjusted for cohort in all pooled analyses to account for unmeasured differences in study populations and protocols that may influence phthalate exposures, childhood adiposity, and covariate values.

Variables Included in Covariate Imputation Models

We ascertained two additional variables in order to predict missing covariate values. For Specific Aim 1, gestational age at delivery was ascertained via medical records and used to

predict maternal last pregnancy weight. For Specific Aim 2, we included gestational age at urine collection as a predictor of urinary creatinine concentration. We calculated gestational age at urine collection by subtracting the number of weeks between urine collection and delivery from the gestational age at delivery (weeks) reported on the medical record.

Variables Included as Predictors of Missing Outcomes

For Specific Aim 2, we included four additional variables reported in a previous study to predict subject retention in the CCEH cohort (Rundle et al. 2012). Receipt of public assistance during pregnancy (yes/no) and maternal satisfaction with living conditions (5 point Likert scale) were ascertained by questionnaire at enrollment. Two neighborhood-level characteristics, poverty rate and Spanish language linguistic isolation, were determined using census data within a 1 kilometer radius of the participant's residence at enrollment.

F. Statistical Methods

Overview

The general statistical approach used in Specific Aims 1 and 2 was similar, and will be described below with additional details where the two studies differed. Our general modeling approach utilized a Bayesian hierarchical framework for fitting linear and logistic mixed effects models with random intercepts to account for multiple observations per child. We selected a Bayesian framework in order to estimate associations while simultaneously accounting for several potential biases, including imputing metabolite concentrations below the LOD (Aim 1), stabilizing estimates from multiple metabolite models (Aims 1 and 2), imputing missing at random covariate data (Aims 1 and 2), and conducting sensitivity analyses exploring potentially informative loss to follow-up (Aims 1 and 2).

Metabolite Concentrations Below the LOD

It has become standard practice to set biomarker values below the LOD to a fixed value. This approach results in appreciable bias when greater than 5-10% of the concentrations are non-detects (Lubin et al. 2004). In the MSSM, CCEH, and HOME studies, all phthalate metabolites except for MEHP were measured above the detection limits in $\geq 95\%$ of urine samples. MEHP is a hydrolytic metabolite of DEHP and was used to calculate the Σ DEHP variable but was not examined on its own. Thus, using a single replacement technique is unlikely to bias effect estimates. In Specific Aim 2, where the values of the LODs differed between cohorts due to changes in CDC laboratory methodologies over time, we set phthalate metabolite concentrations below the LOD to the value of the LOD divided by the square root of two (Hornung and Reed 1990).

In Specific Aim 1, we utilized an alternative approach to account for phthalate metabolite concentrations below the LOD by imputing values from a truncated normal distribution. This approach has been applied in previous studies (Carmichael et al. 2010; Uh et al. 2008). For each metabolite, the parameters of the truncated normal distribution were defined by the mean and SD of the observed distribution, a lower bound of 0, and an upper bound set equal to the LOD. The natural logs of these parameters were used to impute natural log metabolite concentration values at each iteration of the Markov chain Monte Carlo (MCMC) algorithm using the WinBUGS package `djl.trunc.norm`. The Σ DEHP variable was computed using the component metabolite values (MEHP, MEHHP, MECPP, MEOHP) at each MCMC iteration.

Missing at Random Covariate Data

For both Aims, we imputed missing covariate data by including parametric models for variables with missing data within the MCMC procedure under the assumption that data were missing at random. Predictors of missing covariate values were selected on the basis of

observed or expected relationships between each missing covariate and variables measured in the study population.

In Specific Aim 1, we imputed missing values for adequacy of gestational weight gain ($n = 22$), breastfeeding ($n = 1$), and physical activity at follow-up ($n = 3$ children with 8 visits). Missingness in the adequacy of gestational weight gain variable was due to missing last pregnancy weights. Therefore, we included a linear regression model to predict last pregnancy weight on the basis of maternal education, race/ethnicity, sex, smoking during pregnancy, work status during pregnancy, maternal age at delivery, birth weight, maternal height, gestational age, maternal first pregnancy weight, and maternal pre-pregnancy BMI. The predicted last pregnancy weight was then used to calculate adequacy of gestational weight gain at each iteration of the MCMC algorithm, as described in Section E above.

The missing breastfeeding value was multiply imputed using a logistic regression model on the basis of maternal education, race/ethnicity, child's sex, maternal smoking and work status during pregnancy, maternal age at delivery, adequacy of gestational weight gain, maternal pre-pregnancy BMI, and birth weight.

In contrast to the previous two variables that were measured once per child, physical activity was assessed at each follow-up visit. Therefore, we included a logistic mixed effects regression model with random intercepts to predict missing physical activity values at each follow-up visit on the basis of race/ethnicity, maternal pre-pregnancy BMI, maternal smoking during pregnancy, age (in months), birth weight, and child's sex.

For each variable with missing data, we included quadratic or cubic terms for continuous predictors if they exhibited non-linear relationships among those without missing data (evaluated visually using generalized additive models in the SAS procedure PROC GAM).

A similar approach was used to impute missing values in Specific Aim 2. To calculate missing values for pre-pregnancy maternal BMI ($n = 58$) and gestational weight gain ($n = 107$), we included linear regression models for maternal first pregnancy weight, maternal height, and

maternal last pregnancy weight. We used the same set of predictors for these three anthropometric variables: cohort, race/ethnicity, maternal education, parity, maternal smoking and work status during pregnancy, child's sex, maternal age at delivery, and the other maternal anthropometric variables (e.g., for maternal first pregnancy weight, we included maternal height and maternal last pregnancy weight). Linear, quadratic, and cubic terms were included for maternal age and maternal anthropometric variables to allow for non-linear relationships. Maternal pre-pregnancy BMI and gestational weight gain were then calculated using the imputed values at each iteration of the MCMC algorithm. We also included a logistic regression model for missing breastfeeding values ($n = 6$) based on cohort, maternal education, race/ethnicity, parity, and maternal smoking during pregnancy.

Missing Creatinine Concentrations

In Specific Aim 2, we used a similar approach to multiply impute missing creatinine concentrations. As mentioned previously, CCEH measured specific gravity in all urine samples ($n = 339$), with creatinine additionally measured in a subset ($n = 137$). To obtain a common measure of urine dilution, we multiply imputed missing natural log creatinine concentrations in CCEH using a linear regression model at each iteration of the MCMC algorithm in our Bayesian models. Independent variables included specific gravity; gestational age and calendar date at urine collection; maternal education, race/ethnicity, parity, BMI, and height; child's sex; and gestational weight gain. Higher order polynomials were included for specific gravity, gestational age and calendar date at urine collection, and maternal height as they exhibited non-linear associations with creatinine among those with measured values.

As described above (Section C. Exposure Assessment) we constructed creatinine-corrected metabolite concentrations to assess non-linear dose-response relationships. In order to obtain creatinine-corrected metabolite concentrations in the CCEH cohort, we assigned the

posterior mean creatinine concentration predicted by our model to each participant without a creatinine measurement.

We explored the performance of the imputation model by setting a random 25% sample of measured creatinine concentrations to missing and assessing the Spearman correlation coefficient between the true values and posterior mean values predicted by our model ($R^2 = 0.61$). Additionally, we compared assignment of CCEH participants into exposure quartiles using metabolite concentrations corrected for either specific gravity or creatinine. Cohen's weighted Kappa coefficients were similar among those with measured or imputed creatinine concentrations (Table 2). Although this analysis does not directly evaluate the validity of the creatinine prediction model, it indicates that the imputed values agree with specific gravity as well as the measured creatinine concentrations in terms of classifying phthalate metabolite concentrations into quartiles based on urine dilution.

Table 2. Cohen's weighted Kappa coefficient for the agreement of urine dilution-corrected phthalate metabolite quartiles based on either specific gravity or creatinine in the CCEH cohort, comparison of measured and imputed creatinine concentrations

Metabolite	Measured creatinine	Imputed creatinine
MEP	0.57	0.68
MnBP	0.53	0.59
MiBP	0.52	0.59
MCPP	0.52	0.56
MBzP	0.68	0.64
ΣDEHP	0.61	0.61

Bayesian Priors for Multiple Metabolites

Because estimates of association between outcomes and an individual phthalate metabolite may be confounded by other correlated metabolites, we estimated associations in multiple metabolite hierarchical models within our Bayesian framework. The beta coefficient of each standardized phthalate metabolite was given an independent normal prior distribution with a mean of zero and variance of $1/\tau^2$ (MacLehose et al. 2007). We selected values of τ that reflect our prior belief that 95% of the effects of a SD difference in natural log phthalate metabolite concentration are within an odds ratio (OR) of 0.14 to 7.1 for overweight/obese status ($\tau = 1$), or, for continuous outcomes, approximately \pm one SD of the outcome mean in the study population ($\tau = 0.0625$ for percent fat mass, $\tau = 1$ for BMI, weight, and height z-scores).

Covariate Adjustment

We assessed covariates using directed acyclic graphs and adjusted for variables determined to be in the minimally-sufficient adjustment set. For Specific Aim 1, these variables included maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic or other), maternal age at delivery, maternal education ($>$ college degree / \leq college degree), maternal work status during pregnancy (homemaker or student/ employed), maternal pre-pregnancy BMI, maternal height, adequacy of gestational weight gain, maternal smoking during pregnancy (yes/no), breastfeeding (ever/never), calendar date of urine collection, natural log creatinine, child's sex (female/male), and months of age at follow-up. We also included physical activity at follow-up (active/inactive) to improve precision as it was a strong predictor of the outcome. For continuous variables, we included higher order polynomials if they exhibited non-linear associations with percent fat mass.

For Specific Aim 2, we adjusted for cohort, maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, other), maternal age at delivery, maternal education ($<$ high

school, high school or GED, some college, \geq college degree), maternal work status during pregnancy (homemaker or student/employed), maternal pre-pregnancy BMI, maternal height, gestational weight gain, maternal smoking during pregnancy (yes/no), breastfeeding (ever/never), natural log creatinine, calendar date of urine collection, parity (primiparous/multiparous), child's sex (female/male), and months of age at follow-up. In z-score models, we also adjusted for an interaction between child's sex and age at follow-up because outcome distributions by age differed between girls and boys. We included a quadratic term for maternal pre-pregnancy BMI and cubic terms for maternal age and gestational weight gain based on non-linear associations with child body size outcomes.

Outcome Models

For both Specific Aims, we estimated associations in our Bayesian framework using mixed effects models with random intercepts to account for multiple observations per child. For continuous outcomes (percent fat mass and z-scores), we estimated beta coefficients and 95% credible intervals (CI) per SD increase in natural log phthalate metabolite concentrations in linear mixed effects models. For overweight/obese status, we estimated odds ratios (ORs) and 95% CIs per SD increase in natural log phthalate metabolite concentrations in logistic mixed effects models. We ran Bayesian models in WinBUGS version 1.4.3 (MRC Biostatistics Unit, Cambridge, UK) with a 10,000 iteration burn-in followed by 50,000 additional iterations. We assessed model convergence using visual diagnostics (Gelman et al. 2013).

Effect Measure Modification

Due to potential differences in the effects of phthalate metabolites with anti-androgenic activity among girls versus boys, we examined heterogeneity of associations between maternal urinary phthalate concentrations and body size by child's sex in both Specific Aims. As sample

size was limited in Specific Aim 1, we did not evaluate modification by any other variables. In Specific Aim 2, we additionally assessed cohort and race/ethnicity as potential modifiers and further examined heterogeneity of associations by both cohort and child's sex or race/ethnicity.

We assessed effect measure modification in our multiple metabolite models by including interaction terms between the modifier and each metabolite, and between the modifier and natural log creatinine. For Aim 2, models assessing modification by both cohort and either sex or race/ethnicity included all two- and three-way interaction terms between metabolites, cohort, and the covariate. When assessing modification by race/ethnicity in Aim 2, we excluded children of other race as well as Hispanic children in HOME due to small numbers. We considered there to be meaningful effect modification if the 80% CI for the interaction term did not cross the null value.

Non-linear Dose Response

As described in Section C. Exposure Assessment, we categorized exposure into tertiles (Specific Aim 1) or quartiles (Specific Aim 2) based on creatinine-corrected phthalate metabolite concentrations to assess non-linear dose response relationships. For these models, we replaced the linear terms in our models described above with indicator variables for the second and third tertiles (Specific Aim 1) or second through fourth quartiles (Specific Aim 2) of each metabolite and we did not include natural log creatinine in these models.

In Specific Aim 2, we also examined dose-response relationships using restricted quadratic splines with knots at the 20th, 40th, 60th, and 80th percentile of each metabolite or sum. We computed spline variables using the SAS macro RQSMACRO (Howe et al. 2011) and included them in multiple metabolite outcome models using the approach described above. We did not use splines in Specific Aim 1 because we could not combine this method with our approach for imputing concentrations <LOD within the MCMC algorithm.

G. Sensitivity Analyses

Single Metabolite Models

The vast majority of studies examining phthalate exposures in relation to health outcomes estimate associations in separate models for each metabolite. The motivation for this approach is to avoid multicollinearity since phthalate metabolites are correlated. However, this correlation might also lead to confounding of associations with each metabolite by the other metabolites. Therefore, we compared our associations estimated in multiple metabolite models to those in single metabolite models. We specified the same prior distributions for phthalate metabolite beta coefficients as the main analyses but included only one metabolite at a time, e.g. $\beta \sim \text{Normal}(0,1)$.

Potentially Nonignorable Missing Outcomes

Lack of subject retention is a common problem in longitudinal studies and occurred in all three of the study cohorts. Loss to follow-up may result in selection bias since clinic non-attendance may be missing not at random (MNAR). While most studies ignore participant drop out and conduct analyses only among subjects who returned for clinic visits, we explored this potential bias.

For both Aims, our primary analyses of children with at least one follow-up visit assumed that responses were missing at random (MAR) conditional on observed outcomes and covariates. However, there were some minor differences in the distribution of observed variables between the birth cohorts and the children who were followed-up. Furthermore, the probability of a missing body size measure may depend upon its true (unobserved) value. Therefore, we assessed the sensitivity of our findings to potential selection bias using a selection model approach (Little 1995).

For Specific Aim 1, we used a selection model to assess the association between phthalate exposures and percent fat mass among all 380 children with measured prenatal phthalate metabolite concentrations under a nonignorable (MNAR) missing data mechanism. We created a binary missing outcome indicator variable for whether or not percent fat mass was observed at each follow-up visit (0 if observed, 1 if missing). We jointly fit our model for percent fat mass (described above) with a logistic mixed effects model with random intercepts for the binary missing outcome indicator variable. The missing data indicator model was dependent on the (potentially unobserved) percent fat mass value as well as baseline covariates either observed or expected to be associated with loss to follow-up (maternal age, race, work status, pre-pregnancy BMI, and adequacy of gestational weight gain).

We used a very similar approach for Specific Aim 2, though there were differences in 1) the construction of the binary missing outcome indicator variable, and 2) the predictors included in the missing outcome indicator model. In the pooled study, some outcomes were unobserved not because the children were lost to follow-up but because we restricted the study to include outcomes among children observed at ages 4 to 7 years. Thus, we assigned values for the binary missing indicator based on whether or not the child completed a follow-up visit in the scheduled cohort-specific visit window, regardless of whether that outcome was included in our analysis. The logistic mixed effects model for the missing outcome indicator included a large set of predictors including the outcome (potentially unobserved), maternal age, race/ethnicity, education, and pre-pregnancy BMI; child's birth weight and sex; and calendar date of urine collection. In addition, we included interaction terms to allow the beta coefficient for each predictor to vary by cohort. Finally, we included receipt of public assistance during pregnancy, maternal satisfaction with living conditions, neighborhood poverty rate, and Spanish language linguistic isolation in the missing outcome indicator model for members of the CCEH cohort (see section E. Covariates for additional details). We ran a model for each of the four primary outcomes in the pooled cohort (overweight/obese status and BMI, weight, and height z-scores).

For both Aims, we ran ten chains for inference from the MCMC procedure and compared results to models that assumed dropout was missing at random (MAR).

CHAPTER IV. RESULTS FOR SPECIFIC AIM 1

A. Introduction

Childhood obesity rates increased substantially in the past several decades (Ogden and Carroll 2010). In the United States, 16.9% of children aged 2-19 years are obese (Ogden et al. 2014). Compared to normal weight children, obese children are at greater risk of becoming obese adults (Freedman et al. 2005a), with associated risks of diabetes, heart disease, cancer, and many other ailments. Evidence suggests that factors other than energy balance may contribute to the rapid rise in obesity rates (Keith et al. 2006). The concomitant increase in production of synthetic chemicals with rising obesity rates and the recognition that prenatal factors influence childhood obesity have led to a search for environmental obesogens (Baillie-Hamilton 2002; Heindel and vom Saal 2009).

Phthalates, synthetic chemicals with endocrine disrupting properties, are hypothesized to be obesogens. Human exposure to phthalates is ubiquitous, arising from contact with a wide range of consumer products including polyvinyl chloride plastics, building materials, medical devices, pharmaceuticals, toys, food packaging, cosmetics, and fragrances (Schettler 2006). Early life phthalate exposures have been associated with health outcomes in childhood including physical and neurological development, allergic diseases, and anogenital distance in boys (Braun et al. 2013). For some developmental endpoints, phthalate associations have been reported to vary by sex (Engel et al. 2009; Swan et al. 2010; Wolff et al. 2008).

Toxicological evidence suggests that perinatal exposures to phthalates may promote an obesogenic phenotype in offspring through several mechanisms (Grun 2010). Although a number of studies, primarily cross-sectional, have reported associations between urinary

phthalate metabolite concentrations and body size in children (recently reviewed in (Goodman et al. 2014)), findings are inconsistent regarding which phthalates are associated with outcomes or whether associations are positive or inverse. Furthermore, a causal link between phthalate exposures and subsequent body size cannot be established in cross-sectional studies that measure concurrent exposures and outcomes (Engel and Wolff 2013).

Although one small study reported on associations between di-(2-ethylhexyl) phthalate (DEHP) metabolite concentrations measured in cord blood and BMI in the first year of life (de Cock et al. 2014), no previous study has examined gestational exposure, the hypothesized critical window for an effect of phthalates on obesity. To address this gap, we assessed the associations between third trimester urinary phthalate metabolite concentrations and fat mass at ages 4 to 9 years in a prospective birth cohort.

B. Methods

Study population

The Mount Sinai Children's Environmental Health Study enrolled 479 primiparous women with singleton pregnancies from the Mount Sinai Diagnostic and Treatment Center and two adjacent private practices in New York City between 1998 and 2002. Women delivered at the Mount Sinai Medical Center and seventy-five women were subsequently excluded for reasons described elsewhere (Engel et al. 2007). The final cohort consists of 404 infants for whom birth data were available.

Children were invited to return for three follow-up visits scheduled at approximately ages 4-5.5 (mean=4.9), 6 (mean=6.2), and 7-9 (mean=7.8) years, hereafter referred to as visits 1, 2, and 3. Women provided informed consent prior to participation and children aged ≥ 7 years provided assent. The study received approval from the Mount Sinai School of Medicine

Institutional Review Board. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research.

Phthalate exposures

Mothers provided a spot urine sample between 25 and 40 weeks' gestation (mean=31.5 weeks). Samples were analyzed at the CDC laboratory for the following phthalate metabolites: monoethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), monoisobutyl phthalate (MiBP), mono(3-carboxypropyl) phthalate (MCP), monobenzyl phthalate (MBzP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP). Quality control methods have been reported previously (Kato et al. 2005). Correction factors were applied to MBzP (0.72) and MEP (0.66) concentrations and limits of detection (LOD) to adjust for inaccuracies in analytical standards (Centers for Disease Control and Prevention 2012).

Of the 188 children with body composition measurements between ages 4 and 9 years, maternal prenatal phthalate metabolite concentrations were available for 182 children. Following previous work (Wolff et al. 2008), two observations obtained from a very dilute urine (<10 mg/dL creatinine) were excluded from all analyses due to the potential for inaccurate biomarker measurements. Thus, the current analysis includes 180 children (364 total visits). Distributions of prenatal phthalate metabolite concentrations were similar among children with and without a follow-up visit (Engel et al. 2010).

Outcome assessment

At each follow-up visit, we measured children in bare or stocking feet wearing a pediatric gown or light clothing. We assessed weight and body composition via bioelectrical impedance analysis using a pediatric Tanita scale (model TBF-300; Tanita Corporation of America,

Arlington Heights, Illinois). We obtained duplicate measures of height using a stadiometer, and collected a third measure if the difference between the first two measures exceeded 2.0 centimeters.

Using the fat mass estimates reported by the Tanita scale, we calculated percent fat mass as $(\text{fat mass} / \text{weight}) \times 100$. Because the Tanita equations for fat mass have not been validated for children aged <7 years, we tested two alternative equations validated for estimating fat mass from bioelectrical impedance measures in children (Clasey et al. 2011a; Horlick et al. 2002). The Horlick et al. equation did not perform well in this sample (data not shown). Percent fat mass values estimated with the Clasey et al. equation were highly correlated with the Tanita values (Pearson $R^2 = 0.97$) but there were some implausible estimates (e.g., fat free mass > total mass). Thus, we used the Tanita proprietary equations. For supplementary analyses, we also calculated BMI as $\text{weight (kg)} / \text{height (m)}^2$ and determined age- and sex-standardized BMI z-scores using the CDC SAS macro (Centers for Disease Control and Prevention 2004).

Covariates

We collected covariate data from mothers during a two hour structured interview at enrollment. We ascertained pregnancy and delivery characteristics from a computerized perinatal database at Mount Sinai Hospital. We calculated adequacy of gestational weight gain as the ratio of observed gestational weight gain (last pregnancy weight minus self-reported pre-pregnancy weight) to expected gestational weight gain based on the 2009 Institute of Medicine recommendations $\times 100$, and categorized gains as less than recommended (<86%), recommended (86-120%), or more than recommended (>120%) (Bodnar et al. 2010; Bodnar et al. 2011). We classified physical activity at each follow-up visit as active if the parent/caretaker reported the child was “active most of the time” or inactive if the child was “active some of the time” or “hardly at all”.

Statistical analysis

We used a Bayesian modeling framework to assess associations between prenatal phthalate metabolite concentrations and fat mass. We selected this approach in order to estimate associations while simultaneously accounting for several potential biases by (1) imputing metabolite concentrations below the LOD, (2) stabilizing estimates from multiple metabolite models, (3) imputing missing at random covariate data, and (4) accounting for potentially informative loss to follow-up.

First, we accounted for phthalate metabolite concentrations below the LOD by imputing values from a truncated normal distribution, an approach applied in previous studies (Carmichael et al. 2010; Uh et al. 2008). For each metabolite, the parameters of the truncated normal distribution were defined by the mean and standard deviation (SD) of the observed distribution, a lower bound of 0, and an upper bound set equal to the LOD. The natural logs of these parameters were used to impute natural log metabolite concentration values at each iteration of the Markov chain Monte Carlo (MCMC) algorithm using the WinBUGS package `djl.trunc.norm`. Because DEHP metabolites (MECPP, MEHHP, MEHP, MEOHP) represent exposure from the same sources and are highly correlated (Wolff et al. 2008), they were examined as a molar sum (Σ DEHP) that was computed using component metabolite values at each MCMC iteration. The natural log of each phthalate metabolite or sum was standardized to its mean and SD in order to facilitate comparison of relative phthalate effect sizes in relation to their distribution in the study population.

Because estimates of association between fat mass and an individual phthalate metabolite may be confounded by other correlated metabolites, we estimated associations in multiple metabolite hierarchical models within our Bayesian framework. The beta coefficient of each standardized phthalate metabolite was given an independent normal prior distribution with a mean of zero and variance of $1/\tau^2$ (MacLehose et al. 2007). We selected a value of τ that reflects our prior belief that 95% of the effects of a SD difference in natural log phthalate

metabolite concentration are within approximately \pm one SD of the mean percent fat mass in the study population ($\tau = 1/16$). As a sensitivity analysis, we estimated associations using single metabolite models that specified the same prior distributions for phthalate beta coefficients but included only one metabolite at a time.

We adjusted for potential confounding variables identified using directed acyclic graphs. Demographic and socioeconomic characteristics included maternal race/ethnicity, age, education, and work status during pregnancy. We adjusted for maternal body size characteristics (pre-pregnancy BMI, height, and adequacy of gestational weight gain), maternal smoking during pregnancy (yes/no), and breastfeeding (ever/never) to account for early life factors that are associated with childhood overweight status (Weng et al. 2012). We adjusted for calendar date of urine collection to account for temporal trends in phthalate exposure and prevalence of childhood obesity. Additionally, we adjusted for natural log creatinine (to account for urine dilution), child's sex, and months of age at follow-up. We also included physical activity at follow-up (active/inactive) to improve precision as it was a strong predictor of the outcome. Child's sex was evaluated as an effect measure modifier by including an interaction term between sex and each metabolite. Continuous covariates (maternal age, pre-pregnancy BMI, maternal height, adequacy of gestational weight gain, calendar date of urine collection, natural log creatinine, and age at follow-up) were standardized as $(x - \mu_x)/(2\sigma_x)$ to improve MCMC convergence and facilitate comparisons of effect sizes with dichotomous covariates (Gelman et al. 2013). We included higher order polynomials if continuous variables exhibited non-linear associations with percent fat mass. Covariates were given independent null-centered priors with $\tau = 1/64$, reflecting our prior belief that 95% of the effects of binary covariates (or a two SD change in continuous covariates) are within \pm two SDs of the mean percent fat mass in the study population.

Again taking advantage of the Bayesian framework for multiple imputation, we imputed missing at random values for adequacy of gestational weight gain ($n = 22$), breastfeeding ($n = 1$),

and physical activity at follow-up ($n = 3$ children with 8 visits) within the MCMC procedure. Because missing adequacy of gestational weight gain was due to missing last pregnancy weights, we modeled last pregnancy weight as a normally distributed random variable conditional on maternal education, race/ethnicity, child's sex, smoking during pregnancy, work status during pregnancy, maternal age at delivery, birth weight, maternal height, gestational age, maternal first pregnancy weight, and maternal pre-pregnancy BMI. At each iteration of the MCMC algorithm, adequacy of gestational weight gain was calculated using the imputed last pregnancy weight. We modeled breastfeeding using a logistic model conditional on maternal education, race/ethnicity, child's sex, smoking during pregnancy, work status during pregnancy, maternal age at delivery, adequacy of gestational weight gain, maternal pre-pregnancy BMI, and birth weight. We modeled physical activity at each follow-up visit using a logistic mixed effects model with random intercepts, conditional on race/ethnicity, maternal pre-pregnancy BMI, smoking during pregnancy, age at follow-up (months), birth weight, and child's sex. Beta coefficients in the imputation models were given independent, null-centered priors with $\tau = 1$, reflecting our prior belief that 95% of the effects of binary covariates (or a two SD change in continuous covariates) are within \pm two SDs of the mean last pregnancy weight or within an odds ratio of 0.14 to 7.1 for breastfeeding and physical activity.

We assessed associations of third trimester maternal urinary phthalate metabolite concentrations with percent fat mass using linear mixed effects regression models to account for multiple observations per child. We estimated posterior mean beta coefficients and 95% credible intervals (CI) per SD increase in natural log phthalate metabolite concentrations. The interpretation of a CI is more intuitive than that of a confidence interval from a conventional analysis; specifically, given the data and the model, there is a 95% chance that the true value is within this interval. In models examining modification by child's sex, we included interaction terms between sex and each metabolite, as well as between sex and natural log creatinine, and considered there to be meaningful modification if the 80% CI did not cross the null value. To

assess potential non-linear dose-response relationships, we also fit models with indicator variables for the second and third tertile categories of each metabolite. To account for urine dilution in these analyses, we categorized exposure using creatinine-corrected concentrations (micrograms per gram creatinine for metabolites or micromoles per gram creatinine for Σ DEHP).

To assess a second measure of childhood adiposity and facilitate comparisons to studies without body composition data, we also estimated associations between phthalate metabolite concentrations and BMI z-scores using the approach described above.

We ran each of the final Bayesian models for 50,000 iterations after an initial 10,000 iteration burn-in and assessed model convergence using standard diagnostic measures described elsewhere (Gelman et al. 2013). We conducted descriptive analyses in SAS version 9.3 (SAS Institute, Cary, NC) and Bayesian modeling in WinBUGS version 1.4.3 (MRC Biostatistics Unit, Cambridge, UK).

Sensitivity analysis for loss to follow-up

Our primary analyses of children with at least one follow-up visit (N=180) assumed that responses were missing at random conditional on observed outcomes and covariates. Because there were some differences in the distribution of observed variables between the birth cohort and the follow-up sample and because the probability of a missing fat mass measure may depend upon its true (unobserved) value, we assessed the sensitivity of our findings to potential selection bias. We used a selection model approach (Little and Rubin 2002) to model the association between phthalate exposures and percent fat mass among all 380 children with measured prenatal phthalate metabolite concentrations, under a potentially nonignorable (missing not at random) missing data mechanism. For this analysis, we jointly fit our model for percent fat mass with a logistic model for a binary indicator of whether the outcome value was missing at each follow-up visit. The missing data indicator model was dependent on the

(possibly unobserved) percent fat mass value as well as covariates either observed or expected to be associated with loss to follow-up (maternal age at delivery, race/ethnicity, work status during pregnancy, pre-pregnancy BMI, adequacy of gestational weight gain, child's sex). Because results from selection models are sensitive to the assumed missing data mechanism (Little 1995), we explored models with additional covariates in the missing data indicator model (maternal smoking during pregnancy, breastfeeding, maternal education, birth weight, months of age at follow-up) and varied parameterization of continuous variables. Beta coefficients were given null-centered, independent priors with $\tau = 2$ to reflect our prior belief that 95% of the effects of a binary covariate (or a two SD change in continuous covariates) are within an odds ratio of 0.25 to 4. We ran ten chains for inference from the MCMC procedure and compared results to models that assumed dropout was missing at random.

C. Results

Although baseline characteristics of children included in the study sample were similar to those in the birth cohort, there were minor differences (Table 3). For example, children of mothers with younger maternal age, underweight pre-pregnancy BMI, and less than recommended gestational weight gain were more likely to be lost to follow-up. All phthalate metabolites were measured in >90% of third trimester maternal urine samples (Table 4).

Percent fat mass increased with age and differed by child's sex, with a larger SD among girls than among boys at all ages (Table 5). As expected, BMI z-score distributions were less variable as they are age- and sex-standardized (Table 6). Correlations between percent fat mass and BMI z-scores increased with age; Spearman R^2 s were 0.75, 0.82, and 0.91 and the 1st, 2nd, and 3rd visits, respectively.

Estimates of association between standardized natural log phthalate metabolite concentrations and percent fat mass are reported in Table 7. All 95% CIs crossed the null and no associations were modified by child's sex, though estimates were imprecise. In models

assessing metabolite tertiles, there was an inverse dose-response relationship for the association of Σ DEHP with percent fat mass (Figure 2). Compared to the lowest Σ DEHP tertile, fat mass decreased by 1.77 percent (95% CI = -4.48, 0.97) in the middle tertile and by 3.06 percent (95% CI = -5.99, -0.09) in the highest tertile. We found no evidence of associations between tertile categories of other metabolites and percent fat mass (Table 8).

Results of sensitivity analyses are reported in Table 9. Associations estimated in multiple and single metabolite models were similar, though the multiple metabolite model beta coefficients tended to be slightly farther from the null and CIs were less precise. Results of the sensitivity analysis for loss to follow-up indicated that missing fat mass data may indeed be nonignorable; children with more fat mass were less likely to be loss to follow-up. For each 1 percent increase in fat mass, the odds ratio for loss to follow-up was 0.75 (95% CI: 0.67, 0.82). However, associations between phthalate metabolites and percent fat mass were similar to the primary analysis. Varying parameters in the missing data model did not alter results.

Patterns of association between phthalate metabolite concentrations and BMI z-scores were generally consistent with our analyses of percent fat mass, though estimates were attenuated toward the null (Table 10).

D. Discussion

To our knowledge, this is the first prospective study of gestational phthalate exposures and adiposity in childhood. We observed an association of third trimester maternal urinary concentrations of DEHP metabolites with lower percent fat mass among children aged 4 to 9 years. We did not observe notable modification of associations by child's sex though there is limited ability to detect heterogeneity in this small sample. Findings were robust to adjustment for a comprehensive set of confounders and to a sensitivity analysis assessing bias from loss to follow-up.

Phthalate exposures during gestation are hypothesized to affect later life adiposity by modifying peroxisome proliferator activated receptors (PPARs), associated with adipogenesis, lipid and carbohydrate metabolism, and androgen levels, or by disrupting thyroid hormone function (Desvergne et al. 2009; Kim and Park 2014). Our findings suggest an anti-adipogenic effect of DEHP exposure, which is consistent with toxicological studies reporting that relatively high dose DEHP exposure (2% by body weight) induces PPAR alpha-mediated reductions in body weight and fat mass in both rats (Itsuki-Yoneda et al. 2007) and mice (Xie et al. 2002). However, toxicological studies examining early life exposure to DEHP or its hydrolytic metabolite, MEHP, have reported species- and dose-specific effects on subsequent body fat of offspring. Two studies of perinatal MEHP or DEHP exposure in mice reported increased body weight and fat deposition in offspring, though one study observed effects only at the lowest dose administered (0.05 but not 0.25 or 0.5 mg MEHP/kg/day) (Hao et al. 2012) and the other reported effects at both low and high doses (0.05 and 5 mg DEHP/kg/day) (Schmidt et al. 2012). Two studies of pregnant rats exposed to doses ranging from 1 to 400 mg DEHP/kg/day reported no difference in offspring total body weight (Campioli et al. 2014; Kobayashi et al. 2006). Interestingly, Feige et al. reported that although high dose DEHP exposure (500 mg/kg body mass/day) protected against diet-induced obesity via PPAR alpha activation in wild-type mice, mice with humanized PPAR alpha gained more weight and adipose tissue than untreated controls (Feige et al. 2010). While the causes of species differences remain unclear, effects of DEHP on other, less understood pathways (e.g., liver metabolism, thyroid function, or androgen activity) may play a role.

A recent study examined DEHP metabolite concentrations in cord plasma in relation to repeated BMI measurements in the first year of life among 89 infants (de Cock et al. 2014). They report higher BMI among one-year-olds for one metabolite, MEOHP. Although these findings are consistent with the inverse association between Σ DEHP and fat mass observed in our analysis, the de Cock study was based on a small sample at each time point ($n < 10$).

Further, the use of phthalate metabolite concentrations in blood or blood products as exposure biomarkers is questionable (Calafat et al. 2013). While the authors only measured secondary or oxidative metabolites, that may reflect true biological exposures, it is possible that cord plasma DEHP metabolites arise from hospital-based exposures to DEHP at the time of delivery (Vandentorren et al. 2011; Yan et al. 2009).

A number of cross-sectional analyses have examined phthalate exposures and obesity, BMI, waist circumference, or related measures in humans (recently reviewed in (Goodman et al. 2014). Many of these studies reported associations between phthalate metabolites and adiposity-related outcomes, with results often differing between groups defined by age, sex, race/ethnicity, or other characteristics. Such cross-sectional analyses are problematic as it is unclear whether phthalate exposures are causally related to obesity or phthalate body burden differs in obese individuals due to differences in exposure to phthalate sources, such as diet (Serrano et al. 2014), personal care products (Buckley et al. 2012; Duty et al. 2005; Just et al. 2010; Watkins et al. 2014), or medications (Hernandez-Diaz et al. 2013). In addition, these analyses did not assess exposures during gestation, a hypothesized critical window for an effect of phthalates on pathways related to development of obesity.

Phthalates have relatively short half-lives (<24 hours) (Koch et al. 2005) and exposures are likely episodic in nature, so that a single third trimester urine sample may not be representative of exposure during all of gestation. However, fetal growth and adipocyte replication is rapid during the third trimester, indicating that it is a relevant exposure period for fat development (Dietz 1994). Additionally, concentrations of phthalate metabolites in a single urine sample may be moderately predictive of dibutyl, di-iso-butyl, diethyl, and benzylbutyl phthalate exposures during pregnancy, with greater variability for DEHP (Adibi et al. 2008; Braun et al. 2012; Ferguson et al. 2014a).

Although we adjusted for maternal pre-pregnancy BMI and adequacy of gestational weight gain, there may be residual confounding through shared maternal and child diet if the

dietary sources of a woman's phthalate intake during pregnancy are similar to her child's at ages 4 to 9 years. The contribution of diet to total exposure varies by phthalate. Foods typically contain low levels of diethyl phthalate (Serrano et al. 2014) whereas food packaging may account for over half of total DEHP exposure (Rudel et al. 2011). To explain the inverse association we observed between Σ DEHP and percent fat mass, foods that are part of a healthy family diet would also have to contain more DEHP. However, because DEHP has consistently been identified in high fat foods such as dairy, poultry, and cooking oils (Serrano et al. 2014), any confounding by dietary sources of DEHP is more likely in the opposite direction of the association we observed.

While body composition equations built into the Tanita scale are not validated in children under age 7 years, percent fat mass values estimated using an equation validated for this age group was strongly correlated with the values estimated by the proprietary equation. Furthermore, associations between phthalate metabolite concentrations and percent fat mass were consistent across study visits (data not shown) and patterns of association were similar to those for BMI z-score associations. Our sample size was not adequate to examine potential critical windows of susceptibility. Future studies may consider exploring relationships between phthalate exposures and obesity with respect to differing growth trajectories, timing of adiposity rebound, or pubertal onset.

This analysis has several important strengths. First, we examined exposure to phthalates during the hypothesized critical window for developmental programming of obesity. Second, we incorporated repeated measures of fat mass in children. Third, we adjusted for many important confounders including maternal anthropometric characteristics and multiple measures of socioeconomic status. Fourth, we utilized a Bayesian framework to incorporate prior information and account for potential confounding by correlated metabolite concentrations. Finally, we investigated and accounted for missing data in exposures (values <LOD), covariates, and outcomes.

E. Conclusions

In this prospective study, we observed an inverse relation between maternal third trimester concentrations of Σ DEHP metabolites and percent fat mass in children aged 4 to 9 years. While a substantial cross-sectional literature has assessed phthalate metabolite concentrations and obesity-related outcomes, larger prospective studies examining prenatal exposures are needed to replicate these findings.

F. Tables and Figures

Table 3. Characteristics of participants at birth and follow-up, Mount Sinai Children's Environmental Health Study 1998 – 2002

Characteristic	Birth cohort <i>n</i> (%)	Study sample <i>n</i> (%)
Total (N)	404	180
Race/ethnicity		
White	86 (21.3)	34 (18.9)
Black	112 (27.7)	51 (28.3)
Hispanic or other	206 (51)	95 (52.8)
Maternal age at delivery (years)		
< 20	142 (35.2)	56 (31.1)
20–24	132 (32.7)	60 (33.3)
25–29	44 (10.9)	26 (14.4)
≥ 30	86 (21.3)	38 (21.1)
Maternal education (≥ college degree)	100 (24.8)	40 (22.2)
Maternal work status (employed)	235 (58.2)	107 (59.4)
Maternal smoking during pregnancy	67 (16.6)	31 (17.2)
Maternal pre-pregnancy BMI (kg/m ²)		
< 18.5	82 (20.3)	10 (5.6)
18.5–24.9	215 (53.2)	111 (61.7)
25–29.9	72 (17.8)	42 (23.3)
≥ 30	35 (8.7)	17 (9.4)
Maternal height (m) (mean and SD)	1.63 (0.07)	1.62 (0.08)
Adequacy of gestational weight gain		
Less than recommended	44 (12.3)	14 (8.9)
Recommended	75 (21)	40 (25.3)
More than recommended	238 (66.7)	104 (65.8)
Missing	47	22
Year of maternal urine collection		
1998	84 (21.9)	34 (18.9)
1999	127 (33.2)	55 (30.6)
2000	134 (35)	72 (40)
2001	35 (9.1)	18 (10)
2002	3 (0.8)	1 (0.6)
Urine not collected	21	0
Child's sex (male)	222 (55)	98 (54.4)
Breastfed		
Ever	206 (63)	113 (63.1)

Characteristic	Birth cohort <i>n</i> (%)	Study sample <i>n</i> (%)
Never	121 (37)	66 (36.9)
Missing	77	1
Physical activity at follow-up ^a		
Inactive		100 (56.5)
Active most of the time		77 (43.5)
Missing		3

Body mass index (BMI); standard deviation (SD)

^a Proportion classified as inactive at any follow-up visit

Table 4. Distributions of phthalate metabolite concentrations in third trimester maternal urine samples (N=180), Mount Sinai Children's Environmental Health Study 1998 – 2002

Metabolite (µg/L)	LOD	Percent detected	Geometric mean ^a	Minimum	25 th percentile	75 th percentile	Maximum
MEP	0.26	99.4	223	<LOD	87.1	525	29528
MnBP	0.4	100	32.9	0.800	14.3	79.5	4043
MiBP	0.26	97.8	5.83	<LOD	2.90	15.1	76.6
MCPP	0.16	97.8	2.87	<LOD	1.50	6.30	129
MBzP	0.08	99.4	14.1	<LOD	5.70	32.8	481
ΣDEHP ^b	n/a	n/a	0.284	<LOD	0.125	0.530	19.9
MECPP	0.25	99.4	36.0	<LOD	15.1	72.7	2055
MEHHP	0.32	99.4	21.0	<LOD	8.80	41.3	2051
MEHP	0.9	91.7	6.15	<LOD	3.00	14.2	478
MEOHP	0.45	99.4	18.7	<LOD	8.20	38.3	1335

Limits of detection (LOD)

^a To compute the geometric mean, phthalate metabolite concentrations <LOD were replaced by LOD/√2.

^b ΣDEHP is expressed as micromoles/L.

Table 5. Percent fat mass distributions in the Mount Sinai Children's Environmental Health Study 1998 – 2002

Visit	Mean age (years) ± SD	Overall		Girls		Boys	
		<i>n</i>	Mean ± SD	<i>n</i>	Mean ± SD	<i>n</i>	Mean ± SD
All visits	6.5 ± 1.3	363	18.4 ± 8.4	173	17.5 ± 9.8	190	19.3 ± 6.8
Visit 1	4.9 ± 0.4	97	15.3 ± 7.5	47	12.6 ± 8.8	50	17.8 ± 4.8
Visit 2	6.1 ± 0.2	117	17.5 ± 7.2	57	16.4 ± 8.6	60	18.5 ± 5.4
Visit 3	7.8 ± 0.8	149	21.2 ± 9.0	69	21.6 ± 9.8	80	20.9 ± 8.3
Standard deviation (SD)							

Table 6. Age- and sex- standardized body mass index z-score distributions in the Mount Sinai Children's Environmental Health Study 1998 – 2002

Visit	Mean age (years) \pm SD	Overall		Girls		Boys	
		n	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD
All visits	6.5 \pm 1.3	364	0.56 \pm 1.15	174	0.50 \pm 1.10	190	0.62 \pm 1.20
Visit 1	4.9 \pm 0.4	99	0.51 \pm 1.18	49	0.51 \pm 1.07	50	0.52 \pm 1.29
Visit 2	6.1 \pm 0.2	117	0.50 \pm 1.04	57	0.41 \pm 1.04	60	0.59 \pm 1.05
Visit 3	7.8 \pm 0.8	148	0.63 \pm 1.21	68	0.57 \pm 1.17	80	0.69 \pm 1.25
Standard deviation (SD)							

Table 7. Adjusted associations between third trimester maternal urinary phthalate metabolite concentrations and percent fat mass among children aged 4 to 9 years in the Mount Sinai Children's Environmental Health Study 1998 – 2002

Metabolite	Overall	Girls	Boys
MEP	0.12 (-1.34, 1.58)	-0.35 (-2.43, 1.75)	0.75 (-1.31, 2.80)
MnBP	-0.86 (-3.07, 1.36)	-0.34 (-3.71, 3.05)	-0.86 (-3.46, 1.74)
MiBP	0.34 (-1.54, 2.20)	1.04 (-1.36, 3.44)	-0.88 (-3.44, 1.68)
MCPP	0.63 (-1.55, 2.82)	1.21 (-1.44, 3.87)	-0.08 (-3.22, 3.03)
MBzP	0.67 (-1.31, 2.65)	0.62 (-1.77, 3.02)	0.98 (-2.17, 4.14)
Σ DEHP	-0.89 (-2.24, 0.47)	-0.80 (-2.81, 1.23)	-0.64 (-2.46, 1.16)

Beta coefficients (95% credible intervals) per standard deviation increase in natural log phthalate metabolite concentrations were estimated in a multiple metabolite linear mixed effects regression model. Sex-specific estimates were estimated in models including an interaction term for child sex.

Estimates are adjusted for urine dilution and collection date; maternal race/ethnicity, age, education, work status, and smoking during pregnancy; maternal height and pre-pregnancy body mass index; adequacy of gestational weight gain; breastfeeding; months of age and physical activity at follow-up; and, for overall models, child sex.

Figure 2. Adjusted associations between tertiles of third trimester maternal urinary phthalate metabolite concentrations and percent fat mass among children aged 4 to 9 years in the Mount Sinai Children's Environmental Health Study 1998 – 2002

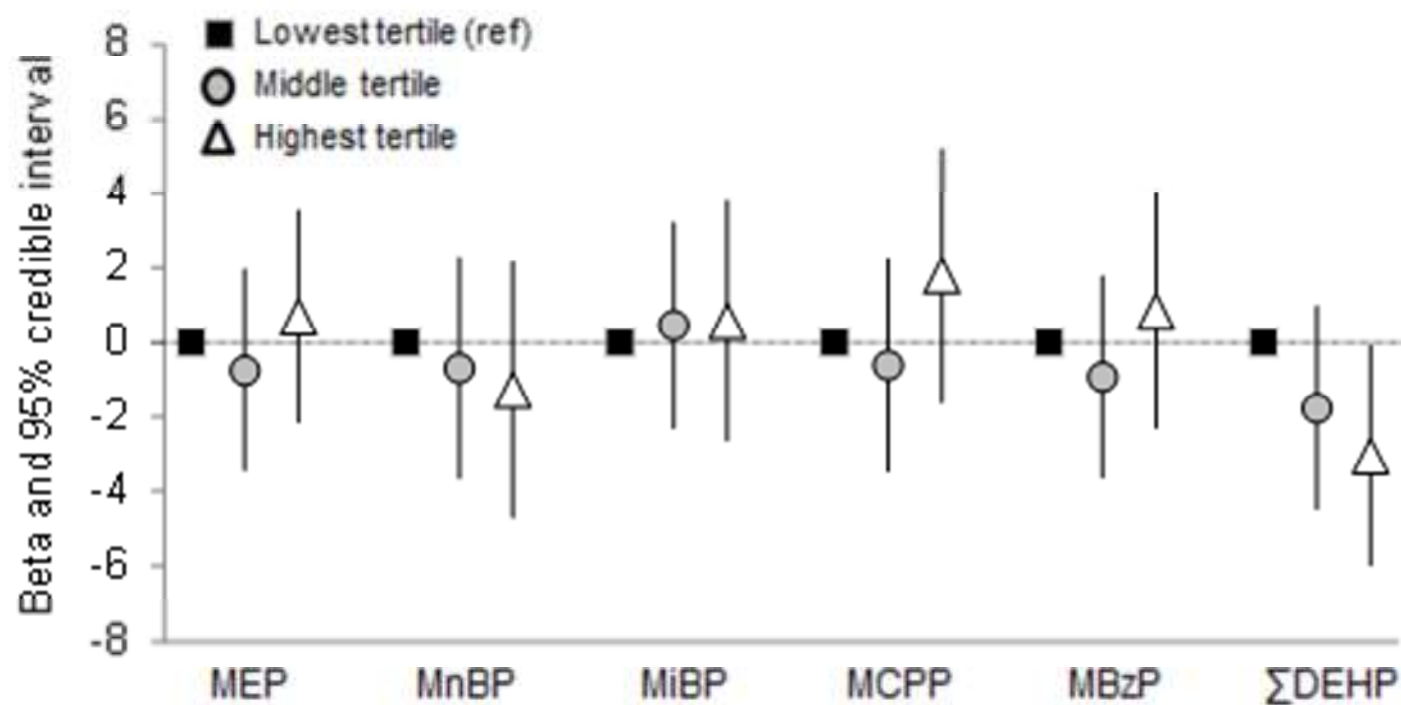


Table 8. Adjusted associations between third trimester maternal urinary phthalate metabolite concentrations and percent fat mass among children aged 4 to 9 years in the Mount Sinai Children's Environmental Health Study 1998 – 2002

Metabolite	Exposure metric	Exposure unit ^a	Overall	Girls	Boys
MEP	Lowest tertile	<186	ref	ref	ref
	Middle tertile	186–546	-0.75 (-3.45, 1.95)	-1.66 (-4.95, 1.69)	-0.20 (-3.81, 3.43)
	Highest tertile	>546	0.69 (-2.15, 3.53)	1.46 (-2.82, 5.73)	2.46 (-1.49, 6.36)
	Continuous	SD	0.12 (-1.34, 1.58)	-0.35 (-2.43, 1.75)	0.75 (-1.31, 2.80)
MnBP	Lowest tertile	<32.0	ref	ref	ref
	Middle tertile	32.0–64.6	-0.69 (-3.66, 2.27)	1.00 (-2.64, 4.60)	-1.80 (-5.68, 2.10)
	Highest tertile	>64.6	-1.27 (-4.69, 2.16)	-2.79 (-7.17, 1.62)	-1.71 (-6.19, 2.82)
	Continuous	SD	-0.86 (-3.07, 1.36)	-0.34 (-3.71, 3.05)	-0.86 (-3.46, 1.74)
MiBP	Lowest tertile	<6.0	ref	ref	ref
	Middle tertile	6.0–12.2	0.46 (-2.30, 3.20)	0.55 (-2.94, 4.02)	0.71 (-2.93, 4.32)
	Highest tertile	>12.2	0.57 (-2.63, 3.79)	0.16 (-4.18, 4.51)	-0.07 (-4.44, 4.32)
	Continuous	SD	0.34 (-1.54, 2.20)	1.04 (-1.36, 3.44)	-0.88 (-3.44, 1.68)
MCPP	Lowest tertile	<2.8	ref	ref	ref
	Middle tertile	2.8–6.2	-0.61 (-3.48, 2.24)	-0.50 (-3.87, 2.90)	-0.72 (-4.60, 3.18)
	Highest tertile	>6.2	1.79 (-1.61, 5.17)	-0.22 (-4.62, 4.17)	1.64 (-2.98, 6.32)
	Continuous	SD	0.63 (-1.55, 2.82)	1.21 (-1.44, 3.87)	-0.08 (-3.22, 3.03)
MBzP	Lowest tertile	<12.7	ref	ref	ref
	Middle tertile	12.7–30.5	-0.93 (-3.65, 1.77)	-2.19 (-5.70, 1.34)	-0.72 (-4.35, 2.92)
	Highest tertile	>30.5	0.84 (-2.29, 4.01)	1.48 (-2.99, 5.93)	-0.79 (-5.28, 3.74)
	Continuous	SD	0.67 (-1.31, 2.65)	0.62 (-1.77, 3.02)	0.98 (-2.17, 4.14)
ΣDEHP	Lowest tertile	<0.23	ref	ref	ref
	Middle tertile	0.23–0.60	-1.77 (-4.48, 0.97)	-1.53 (-4.96, 1.88)	-1.79 (-5.34, 1.75)
	Highest tertile	>0.60	-3.06 (-5.99, -0.09)	-0.27 (-4.51, 4.00)	-2.99 (-7.10, 1.15)
	Continuous	SD	-0.89 (-2.24, 0.47)	-0.80 (-2.81, 1.23)	-0.64 (-2.46, 1.16)

Referent category (ref), standard deviation (SD)

^a Tertile cut points are expressed as micrograms per gram creatinine (or micromoles per gram creatinine for Σ DEHP)

Beta coefficients (95% credible intervals) were estimated in multiple metabolite linear mixed effects regression models adjusted for urine collection date; maternal race/ethnicity, age, education, work status, and smoking during pregnancy; maternal height and pre-pregnancy body mass index; adequacy of gestational weight gain; breastfeeding; months of age and physical activity at follow-up; and, for overall models, child sex. Models of continuous metabolite concentrations were additionally adjusted for natural log creatinine. Sex-specific estimates were estimated in models including an interaction term between child sex and variables for each metabolite or Σ DEHP.

Table 9. Sensitivity analyses for associations between third trimester maternal urinary phthalate metabolite concentrations and percent fat mass among children aged 4 to 9 years in the Mount Sinai Children's Environmental Health Study 1998 – 2002

Metabolite	Primary analysis ^a	Single metabolite models ^b	Loss to follow-up ^c
MEP	0.12 (-1.34, 1.58)	0.08 (-1.14, 1.30)	-0.33 (-1.82, 1.15)
MnBP	-0.86 (-3.07, 1.36)	-0.07 (-1.41, 1.27)	-0.96 (-3.24, 1.31)
MiBP	0.34 (-1.54, 2.20)	0.23 (-1.12, 1.52)	1.24 (-0.70, 3.19)
MCPP	0.63 (-1.55, 2.82)	0.30 (-1.05, 1.66)	0.26 (-1.99, 2.48)
MBzP	0.67 (-1.31, 2.65)	0.40 (-0.93, 1.74)	0.49 (-1.48, 2.49)
Σ DEHP	-0.89 (-2.24, 0.47)	-0.57 (-1.79, 0.57)	-0.47 (-1.90, 0.96)

Beta coefficients (95% credible intervals) per SD increase in natural log phthalate metabolite concentrations, adjusted for urine dilution and collection date; maternal race/ethnicity, age, education, work status, and smoking during pregnancy; maternal height and pre-pregnancy body mass index; adequacy of gestational weight gain; breastfeeding; months of age and physical activity at follow-up; and child sex.

^a Associations among children with at least one follow-up visit (N=180) estimated in a multiple metabolite linear mixed effects regression model.

^b Associations among children with at least one follow-up visit (N = 180) estimated in a separate linear mixed effects regression model for each metabolite.

^c Associations among all children in the birth cohort with measured phthalate metabolite concentrations (N = 380) estimated in a multiple metabolite linear mixed effects regression model using a selection model for potentially nonignorable missing outcome data.

Table 10. Adjusted associations between third trimester maternal urinary phthalate metabolite concentrations and age- and sex- standardized body mass index z-scores among children aged 4 to 9 years in the Mount Sinai Children's Environmental Health Study 1998 – 2002

Metabolite	Exposure metric	Exposure unit ^a	Overall		
			Girls	Boys	
MEP	Lowest tertile	<186	ref	ref	ref
	Middle tertile	186–546	0.16 (-0.23, 0.55)	-0.02 (-0.48, 0.44)	0.34 (-0.18, 0.87)
	Highest tertile	>546	0.17 (-0.24, 0.58)	0.13 (-0.35, 0.61)	0.23 (-0.33, 0.79)
	Continuous	SD	0.05 (-0.16, 0.27)	0.02 (-0.28, 0.33)	0.15 (-0.15, 0.45)
MnBP	Lowest tertile	<32.0	ref	ref	ref
	Middle tertile	32.0–64.6	-0.17 (-0.59, 0.25)	0.07 (-0.43, 0.57)	-0.41 (-0.96, 0.15)
	Highest tertile	>64.6	-0.19 (-0.67, 0.30)	-0.16 (-0.69, 0.38)	-0.25 (-0.90, 0.39)
	Continuous	SD	-0.13 (-0.45, 0.19)	-0.10 (-0.57, 0.37)	-0.15 (-0.53, 0.23)
MiBP	Lowest tertile	<6.0	ref	ref	ref
	Middle tertile	6.0–12.2	0.10 (-0.30, 0.50)	0.16 (-0.33, 0.65)	0.05 (-0.47, 0.57)
	Highest tertile	>12.2	0.03 (-0.42, 0.49)	0.06 (-0.44, 0.57)	-0.05 (-0.68, 0.58)
	Continuous	SD	-0.02 (-0.29, 0.25)	0.04 (-0.30, 0.38)	-0.13 (-0.51, 0.24)
MCP	Lowest tertile	<2.8	ref	ref	ref
	Middle tertile	2.8-6.2	0.11 (-0.31, 0.52)	0.11 (-0.37, 0.59)	0.19 (-0.37, 0.74)
	Highest tertile	>6.2	0.32 (-0.16, 0.79)	0.35 (-0.18, 0.87)	0.40 (-0.26, 1.05)
	Continuous	SD	0.14 (-0.17, 0.46)	0.16 (-0.22, 0.54)	0.16 (-0.29, 0.61)
MBzP	Lowest tertile	<12.7	ref	ref	ref
	Middle tertile	12.7–30.5	-0.18 (-0.56, 0.22)	-0.23 (-0.72, 0.26)	-0.23 (-0.75, 0.29)
	Highest tertile	>30.5	-0.20 (-0.65, 0.25)	-0.06 (-0.56, 0.44)	-0.38 (-1.02, 0.26)
	Continuous	SD	-0.02 (-0.32, 0.27)	0.03 (-0.33, 0.38)	-0.11 (-0.56, 0.34)
ΣDEHP	Lowest tertile	<0.23	ref	ref	ref
	Middle tertile	0.23–0.60	-0.10 (-0.49, 0.29)	-0.17 (-0.65, 0.30)	0.01 (-0.50, 0.52)
	Highest tertile	>0.60	-0.13 (-0.55, 0.29)	-0.13 (-0.60, 0.35)	-0.08 (-0.66, 0.51)

Continuous	SD	-0.05 (-0.25, 0.15)	-0.05 (-0.34, 0.24)	-0.01 (-0.28, 0.26)
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Referent category (ref), standard deviation (SD)

^a Tertile cut points are expressed as micrograms per gram creatinine (or micromoles per gram creatinine for Σ DEHP)

Beta coefficients (95% credible intervals) were estimated in multiple metabolite linear mixed effects regression models adjusted for urine collection date; maternal race/ethnicity, age, education, work status, and smoking during pregnancy; maternal height and pre-pregnancy body mass index; adequacy of gestational weight gain; breastfeeding; months of age and physical activity at follow-up; and, for overall models, child sex. Models of continuous metabolite concentrations were additionally adjusted for natural log creatinine. Sex-specific estimates were estimated in models including an interaction term between child sex and variables for each metabolite or Σ DEHP.

CHAPTER V. RESULTS FOR SPECIFIC AIM 2

A. Introduction

One out of three children aged 2 to 19 years in the United States is overweight or obese (Ogden et al. 2014). Obese children have poorer physical and psychosocial health compared to their normal-weight peers (Daniels et al. 2005) and exhibit early physiologic changes associated with chronic health conditions such as cardiovascular disease (Friedemann et al. 2012). While energy intake is a key determinant of weight change, the “environmental obesogen hypothesis” posits that prenatal environmental chemical exposures may have contributed to the rise in obesity in the last several decades (Grun and Blumberg 2006).

Phthalates are industrial chemicals used as plasticizers and solvents in consumer goods and personal care products (Schettler 2006). Biomonitoring studies have reported widespread exposure to phthalates in the United States (Centers for Disease Control and Prevention 2014) and prenatal exposures to certain phthalates have been associated with reproductive and developmental outcomes in children (Braun et al. 2013). Furthermore, early life phthalate exposures may alter metabolic and homeostatic mechanisms related to the development of obesity (Grun and Blumberg 2006). For example, toxicological studies have demonstrated that certain phthalates affect thyroid or steroid hormone levels and interfere with peroxisome proliferator-activated receptors (PPARs), which regulate lipid metabolism and adipogenesis (Grun and Blumberg 2009; Hatch et al. 2010).

Two prospective investigations of prenatal (Buckley et al. submitted) or intrapartum (de Cock et al. 2014) phthalate exposures reported negative associations of di-(2-ethylhexyl) phthalate (DEHP) metabolites with fat mass or body mass index (BMI) in children. However,

these studies were small with limited ability to evaluate the relation between phthalate exposures and adiposity or assess potential differences in associations within population subgroups. We examined associations of prenatal urinary phthalate metabolite concentrations and BMI assessed in children between ages 4 and 7 years in three cohort studies in the United States.

B. Methods

Children's Environmental Health Center cohorts

The Mount Sinai School of Medicine Center for Children's Environmental Health (MSSM) enrolled 479 primiparous women with singleton pregnancies from the Mount Sinai prenatal clinic and two adjacent private practices in New York City between 1998 and 2002. Women delivered at the Mount Sinai Medical Center. Seventy-five women were subsequently excluded for reasons described elsewhere (Engel et al. 2007). The final cohort consists of 404 mother-infant pairs for whom birth data were available.

The Columbia Center for Children's Environmental Health (CCEH) enrolled 727 pregnant women between 1998 and 2006. The cohort was restricted to non-smoking women 18-35 years old who self-identified as either African American or Dominican and who had resided in Northern Manhattan or the South Bronx in New York City for >1 year prior to pregnancy. Additional details of the study population have been previously reported (Perera et al. 2003; Whyatt et al. 2003).

The Health Outcomes and Measures of the Environment (HOME) Study, a prospective birth cohort located in Cincinnati, Ohio, enrolled 468 women between 2003 and 2006. Because the HOME Study contains a nested, randomized trial of in-home lead and injury hazard controls women had to be living in housing built before 1978. Additional eligibility criteria and study population characteristics have been described (Braun et al. 2009). A total of 389 women delivered live-born, singleton infants without birth defects.

Questionnaires were administered to each mother at study enrollment to ascertain maternal characteristics including age at delivery, race/ethnicity, education, work status during pregnancy, parity, height, and pre-pregnancy BMI. We calculated gestational weight gain as last pregnancy weight minus self-reported pre-pregnancy weight. Women provided a spot urine sample at mean \pm standard deviation (SD) gestational ages of 31.6 ± 5.1 (MSSM), 34.4 ± 3.0 (CCEH), and 27.1 ± 2.2 (HOME) weeks. Child's sex was ascertained from birth records and breastfeeding was assessed by questionnaire at follow-up.

For MSSM, we determined maternal smoking during pregnancy based on self-report. For HOME, we classified women as active smokers during pregnancy if the average of three cotinine concentrations (measured at 16 weeks, 26 week, and birth) exceeded 3 ng/mL (Benowitz et al. 2009). CCEH excluded women with evidence of active smoking (Whyatt et al. 2003).

Human subjects

Women provided informed consent prior to participation and children aged ≥ 7 years provided assent. The MSSM, CCEH, and HOME studies received approval from the Institutional Review Boards of the Mount Sinai School of Medicine, Columbia University, and the University of Cincinnati College of Medicine, respectively. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research.

Phthalate exposures

All spot urine samples were analyzed by CDC for monoethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), monoisobutyl phthalate (MiBP), mono(3-carboxypropyl) phthalate (MCP), monobenzyl phthalate (MBzP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-

hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP). Sample collection and storage practices, analytic methods, and quality control procedures have been described (Kato et al. 2005; Silva et al. 2007). We replaced values below the LOD by the LOD divided by the square root of two for all analyses (Hornung and Reed 1990; Perkins et al. 2007). Following previous work (Wolff et al. 2008), we examined DEHP metabolites (MECPP, MEHHP, MEHP, MEOHP) as a micromolar sum (Σ DEHP, micromoles per liter). To facilitate comparison of phthalate effect sizes, we standardized the natural log concentration of each phthalate metabolite to its mean and SD in the pooled sample.

Outcome assessment

Weight and height were measured at follow-up visits scheduled for approximately ages 4-5.5, 6, and 7-9 years (MSSM), 5 and 7 years (CCEH), and 4, 5, and 7-9 years (HOME). We measured children in bare or stocking feet wearing light clothing (i.e., pediatric gown, underwear, or shorts and a t-shirt). We assessed weight using a digital scale (HOME and CCEH 5 year visit) or a pediatric Tanita scale (MSSM and CCEH 7 year visit) (models TBF-300 and BC-418, Tanita Corporation of America, Arlington Heights, Illinois) and determined height using wall-mounted stadiometers. We calculated BMI [$\text{weight (kg)} / \text{height (m)}^2$] as a measure of excess weight for height that is a moderately sensitive and highly specific indicator of adiposity (Freedman and Sherry 2009). We computed age- and sex-standardized z-scores and percentiles for BMI using a CDC macro (Centers for Disease Control and Prevention 2004). We then classified children as overweight or obese at each follow-up visit if their age- and sex-standardized BMI percentile exceeded 85. As supplementary outcomes, we also calculated age- and sex-standardized weight and height z-scores using the CDC macro (Centers for Disease Control and Prevention 2004).

Statistical analysis

The current analysis includes infants of singleton pregnancies born at ≥ 32 weeks gestation and ≥ 1500 grams with measured maternal urinary phthalate concentrations. After excluding samples with a very dilute urine concentration (< 10 mg/dL creatinine, $n = 9$), (Wolff et al. 2008) 1143 children met our baseline inclusion criteria. To examine BMI prior to puberty, we further restricted the sample to children with weight and height data collected at one or more follow-up visits occurring between 4 and 7 years of age (707 children with 1416 follow-up visits).

We assessed associations of prenatal phthalate exposures with overweight/obesity and BMI z-scores using a Bayesian modeling framework to (1) account for missing at random covariate data, (2) stabilize estimates of correlated metabolites, and (3) conduct sensitivity analyses for potentially nonignorable loss to follow-up.

We identified potential confounders using directed acyclic graphs and adjusted for cohort, maternal race/ethnicity, maternal age at delivery, maternal education, maternal work status during pregnancy, maternal pre-pregnancy BMI, maternal height, gestational weight gain, maternal smoking during pregnancy, calendar date of urine collection, parity, child's sex, breastfeeding, and months of age at follow-up. To account for urine dilution in models assessing continuous phthalate concentrations, natural log creatinine was included as a covariate (Barr et al. 2005). In z-score models, we also adjusted for an interaction between child's sex and age at follow-up because outcome distributions by age differed between girls and boys. We included a quadratic term for maternal pre-pregnancy BMI and cubic terms for maternal age and gestational weight gain based on non-linear associations with outcomes.

MSSM and HOME measured creatinine to account for urinary dilution. CCEH measured specific gravity in all urine samples ($n = 339$), with creatinine additionally measured in a subset ($n = 137$). To obtain a common measure of urine dilution, we multiply imputed missing natural log creatinine concentrations in CCEH using a linear regression model at each iteration of the MCMC algorithm in our Bayesian models. We modeled natural log creatinine as a normally

distributed random variable conditional on specific gravity; gestational age and calendar date at urine collection; maternal education, race/ethnicity, parity, BMI, and height; child's sex; and gestational weight gain.

We also multiply imputed missing at random values of breastfeeding ($n = 6$), maternal pre-pregnancy BMI ($n = 58$), and gestational weight gain ($n = 107$) within our Bayesian framework. We modeled breastfeeding using a logistic model conditional on cohort, maternal education, race/ethnicity, parity, and maternal smoking during pregnancy. We modeled maternal pre-pregnancy weight, maternal last pregnancy weight, and maternal height as normally distributed random variables conditional on cohort, maternal education, race/ethnicity, parity, maternal smoking during pregnancy, maternal work status during pregnancy, child's sex, and maternal age at delivery. At each iteration of the MCMC algorithm, maternal pre-pregnancy BMI and gestational weight gain were calculated based on these imputed values.

To account for potential confounding among correlated phthalate metabolites, we estimated associations in multiple metabolite Bayesian hierarchical models (MacLehose et al. 2007). Following standard practice, the beta coefficient for each standardized phthalate metabolite was given an independent normally-distributed prior distribution with a mean of zero and variance of $1/\tau^2$. We set $\tau = 1$, which represents a prior belief that 95% of the effects of a SD difference in natural log phthalate metabolite concentration are within an odds ratio (OR) of 0.14 to 7.1 for overweight/obese status or approximately \pm one SD of the mean BMI, weight, and height z-score distribution in the study sample.

We estimated posterior mean ORs and beta coefficients, and associated 95% credible intervals (CI), per SD increase in natural log phthalate metabolite concentrations in logistic and linear mixed effects models with random intercepts to account for multiple observations per child. We examined effect measure modification by cohort, child's sex, and race/ethnicity by including interaction terms between the modifier and each metabolite. In models assessing modification by both cohort and either sex or race/ethnicity, we included all two- and three-way

interaction terms between metabolites, cohort, and the covariate. When assessing modification by race/ethnicity, we excluded children of other race as well as Hispanic children in HOME due to small numbers. We considered there to be meaningful effect modification if the 80% CI for the interaction term did not cross the null value. Finally, we explored dose-response relationships in models that replaced continuous variables for standardized metabolite concentrations with restricted quadratic splines (Howe et al. 2011).

As a sensitivity analysis, we compared associations estimated in multiple metabolite models to those estimated in models including only one metabolite at a time, a common approach that may result in confounding by correlated metabolites. Single metabolite models specified the same prior distributions for phthalate beta coefficients as the main analyses, i.e. $\beta \sim N(0,1)$.

Additionally, we conducted a sensitivity analysis for loss to follow-up due to concern that the probability of follow-up visit attendance may depend on a child's outcome value at the time. For this analysis, we compared results from our primary models, which assume outcome data are missing at random, to a selection model approach (Little and Rubin 2002) that assumes a potentially nonignorable (missing not at random) missing data mechanism. We constructed a binary missing outcome indicator variable for attendance at each follow-up visit based on the cohort-specific visit schedules (0 if observed, 1 if missing). Within our MCMC algorithm, we jointly fit the outcome model with cohort-specific models for the missing outcome indicator to allow predictors of missingness to vary by cohort. We specified the missing outcome indicator models as logistic mixed effects regression models with random intercepts, dependent on the outcome (potentially unobserved) as well as maternal age, race/ethnicity, maternal education, maternal pre-pregnancy BMI, birth weight, child's sex, and calendar date of urine collection. The CCEH missing outcome indicator model additionally included four variables that were unmeasured in the other cohorts but previously reported to predict subject retention in CCEH (receipt of public assistance during pregnancy, maternal satisfaction with living conditions,

neighborhood poverty rate, Spanish language linguistic isolation) (Rundle et al. 2012). Finally, because selection models are sensitive to unverifiable assumptions underlying the specification of the missing data model (Little 1995), we varied the parameterization of the missing outcome indicator models by using different functional forms for continuous variables, fitting a single missing indicator model for all three cohorts, and including interaction terms between age at follow-up and child's sex and race/ethnicity.

We conducted descriptive analyses in SAS version 9.3 (SAS Institute, Cary, NC). We ran Bayesian models in WinBUGS version 1.4.3 (MRC Biostatistics Unit, Cambridge, UK) with a 10,000 iteration burn-in followed by 50,000 additional iterations. We ran ten chains for inference from the MCMC procedure for our sensitivity analyses for nonignorable missing data. We assessed model convergence using standard diagnostic measures (Gelman et al. 2013).

C. Results

Distributions of participant characteristics by cohort illustrate differences in the inclusion criteria and source populations of each study (Table 11). For example, HOME enrolled primarily white, non-Hispanic mothers who were older and had more years of education than women in the New York City cohorts. Within each cohort, characteristics of the study sample were similar to those of the eligible population at birth (Table 12).

Except for MEHP, a hydrolytic metabolite of DEHP, urinary phthalate metabolite concentrations were measured in $\geq 95\%$ of urine samples (Table 13). Although geometric mean urinary phthalate metabolite concentrations were lower in HOME than among MSSM and CCEH participants, exposure distributions exhibited substantial overlap (Table 13). Children in HOME were also leaner than children in the other two cohorts. Notably, HOME participants were classified as overweight or obese at 19% of follow-up visits compared to 32% in MSSM and

41% in CCEH (Table 14). Similarly, mean age- and sex- standardized BMI z-scores were highest among CCEH children and lowest in HOME (Table 15).

Adjusted associations of prenatal urinary phthalate concentrations with overweight/obese status and age- and sex-standardized BMI z-scores are reported in Table 16 (overall and by child's sex) and Table 17 (by race/ethnicity). MCPP was associated with increased odds of overweight/obese status, with a stronger magnitude of association among Hispanic children. MCPP was not associated with age- and sex- standardized BMI z-scores. We observed modification of the association between MEP and BMI z-scores by child's sex, with a negative association among girls but not boys (Table 16).

In supplementary analyses of age- and sex-standardized weight and height z-scores, MEP was negatively associated with weight and height z-scores among girls (Table 18). We observed a negative association of MBzP with weight and height z-scores among non-Hispanic black children only (Table 19). We did not observe any associations between MnBP, MiBP, or Σ DEHP and overweight/obese status or BMI, weight, or height z-scores.

Spline models assessing the shape of relationships between prenatal urinary phthalate metabolite concentrations and outcomes were consistent with null associations or a linear dose-response trend for all metabolites and outcomes (data not shown).

Cohort-specific associations are reported in Tables 20-23. Associations with overweight/obese status did not differ by cohort. Associations of two metabolites, MnBP and MEP, with BMI z-scores were modified by cohort such that beta coefficients were on the opposite side of the null in MSSM compared to CCEH and HOME. However, MSSM was the smallest cohort and CIs were imprecise.

Associations were similar whether estimated in single or multiple metabolite models (Table 24). Our sensitivity analysis for loss to follow-up indicated that children with higher BMI z-scores were more likely to be observed. However, estimates of association between prenatal phthalate concentrations and overweight/obese status and BMI z-scores were similar whether

or not we accounted for potentially nonignorable missing outcomes (Table 24). Findings were similar in models with alternative specifications of the missing outcome indicator model (data not shown).

D. Discussion

In this pooled study of three birth cohorts, each SD increase in prenatal urinary MCPP concentrations was associated with greater than twice the odds of overweight or obesity among 4 to 7 year old children. We also observed a negative association of prenatal MEP concentrations with BMI z-scores among girls.

Two prospective studies have assessed early life phthalate exposures and childhood adiposity, one small study utilizing intrapartum cord blood measures of DEHP metabolites (de Cock et al. 2014) and a previous analysis of the MSSM cohort in which third trimester urinary concentrations of both high and low molecular weight phthalate metabolites were assessed (Buckley et al. submitted). In the first study, low cord blood MEOHP concentrations were associated with greater BMI among infant boys (de Cock et al. 2014). However, intrapartum phthalate concentrations may be influenced by medically-related exposures occurring during delivery that cannot be generalized to the antecedent prenatal period. In the previous MSSM study (Buckley et al. submitted), children in the highest tertile of Σ DEHP concentrations had lower percent fat mass than children in the first tertile. Other metabolites were not associated with percent fat mass in children. In the current study, we did not observe associations of Σ DEHP metabolites with outcomes, though there were weak negative associations in some subgroups. It is possible that associations of Σ DEHP with body fatness are not detectable using BMI measurements that do not distinguish between fat and lean mass.

We observed an association of MCPP with increased odds of overweight/obese status but not with differences in BMI z-scores. In children, use of BMI cut points to identify those with excess body fat is moderately sensitive and highly specific (Freedman and Sherry 2009).

Consequently, the association of MCPP with overweight/obese status but not BMI z-scores may reflect that BMI is a less sensitive marker of fat mass. While toxicological literature investigating DnOP is sparse, it does not appear to affect estrogen (Chen et al. 2014) or androgen (Saillenfait et al. 2011) activity but may impact the thyroid and liver (Poon et al. 1997). Consistent with our findings, a study of first trimester urinary phthalate metabolite concentrations and cord blood levels of two adipocyte-produced hormones reported associations of MCPP, but not other phthalate metabolites, with leptin and adiponectin levels (Ashley-Martin et al. 2014). Male infants in the second, third, and fourth quartiles of MCPP exposure had increased odds of high ($\geq 90^{\text{th}}$ percentile) leptin and females in the highest quartile of MCPP exposure had higher odds of high ($\geq 90^{\text{th}}$ percentile) adiponectin.

MCPP is a metabolite of di-*n*-octyl phthalate (DnOP), which, like DEHP, is a high molecular weight phthalate used in plastics and food packaging (National Science Foundation 2008). Both MCPP and DEHP have been associated with consumption of meat, dairy, and other fatty foods (Serrano et al. 2014). We lacked information on diet and cannot rule out confounding by dietary sources of a woman's phthalate exposures that may also be related to obesity in her child. The strongest predictor of childhood overweight is maternal overweight (Salsberry and Reagan 2005), and we adjusted for maternal pre-pregnancy BMI and gestational weight gain to control for potential confounding through shared maternal and child diet. Furthermore, the inverse association of Σ DEHP concentrations with overweight/obese status provides indirect evidence that associations are not strongly confounded by diet since Σ DEHP and MCPP exposures are associated with intake of similar foods (Serrano et al. 2014).

We evaluated heterogeneity of associations by child's sex because certain phthalates exhibit anti-androgenic activity (National Science Foundation 2008). The metabolite for which we observed sexual dimorphism, MEP, does not produce anti-androgen effects in animal studies (National Science Foundation 2008). It has been asserted that associations of MEP with outcomes related to androgen insufficiency in human studies may be due to its correlation with

other phthalates (National Science Foundation 2008). Our associations were co-adjusted but could have been confounded by phthalates not measured in this study or other endocrine disrupting chemicals contained in personal care products. Alternatively, MEP may be related to sex differences in other pathways related to development, such as effects on thyroid hormones. Thyroid dysfunction is more common among women, potentially due to estrogen-mediated differences in oxidative stress (Fortunato et al. 2014).

We restricted our analysis to early childhood because phthalate exposures may affect puberty (Ferguson et al. 2014b; Wolff et al. 2014), a developmental stage that is also strongly tied to BMI. Similar to our finding that MEP concentrations were associated with decreased BMI z-scores among girls, Harley et al. reported an inverse association of prenatal urinary BPA concentrations with BMI and body fat among girls only, and this association was stronger among girls who had not yet reached puberty (Harley et al. 2013). Evaluating associations among older children is warranted to determine whether associations persist with age and to evaluate the timing of potential obesogenic effects with respect to puberty.

Associations by race/ethnicity must be interpreted with caution. Because over 80% of the non-Hispanic white children in the study sample were HOME Study participants, differences in associations among white compared to black or Hispanic children may reflect differences in unmeasured characteristics of the HOME population. In particular, HOME Study mothers were older and more educated and their children were leaner compared to the other two cohorts, leading to potential differences in the magnitude of residual confounding in the HOME study as well as susceptibility to obesogenic effects. Hispanic participants in MSSM were primarily of Puerto Rican origin whereas CCEH Hispanics were Dominican. Although estimates of association among Hispanic children in MSSM and CCEH were similar for overweight/obese status, there were differences in some associations with z-score outcomes. Cohort-specific estimates among non-Hispanic black children, who were included in all three studies, were more comparable. For example, the negative association of MBzP and height z-scores among

non-Hispanic black children was observed at a similar magnitude in each of the three cohorts (β = -0.38, -0.32, -0.40 in MSSM, CCEH, and HOME, respectively).

We measured phthalate metabolite concentrations in a single spot urine sample, which may not represent exposures throughout pregnancy since phthalates are quickly eliminated from the body (<24 hours) (Koch et al. 2005) and exposure to phthalate sources is episodic. However, many sources of phthalate exposure are frequent and may result in steady-state levels (Calafat and McKee 2006; Hauser et al. 2004). The probability of correctly classifying “high exposure” over a 6-12 week period using a single urine sample was reported in two studies to range between 0.50 and 0.74, depending on the metabolite (Adibi et al. 2008; Hauser et al. 2004).

Despite limitations noted above, this prospective study has several important strengths. We assessed exposures during the fetal period, which is thought to be a susceptible developmental window for the origins of obesity (Dietz 1994). Assessing associations in three independent cohorts with notable variation in population characteristics supports the robustness of our findings. Pooling the cohorts provided a large sample size to assess heterogeneity of associations by hypothesized modifying factors. We employed a flexible Bayesian modeling approach that accommodated methods to multiply impute missing covariate data and control for confounding among correlated metabolites. Finally, we conducted sensitivity analyses to explore potential bias from loss to follow-up.

E. Conclusions

We observed a positive association of prenatal urinary MCPP concentrations with overweight/obese status in children aged 4 to 7 years. Our findings do not suggest that prenatal exposures to other phthalates are obesogenic. We observed a negative association of MEP with BMI z-scores among girls, indicating that MEP may interfere with physical development though

we cannot rule out confounding (e.g., other environmental obesogens) as an alternative explanation.

F. Tables and Figures

Table 11. Characteristics of the study sample at baseline by cohort and pooled

Characteristic	MSSM	CCEH	HOME	Pooled
Total (N)	151	339	217	707
Maternal age at delivery (years)				
< 20	42 (28)	47 (14)	13 (6)	102 (14)
20–24	53 (35)	121 (36)	37 (17)	211 (30)
25–29	21 (14)	100 (30)	62 (29)	183 (26)
≥ 30	35 (23)	71 (21)	105 (48)	211 (30)
Race/ethnicity				
Non-Hispanic white	31 (21)	0 (0)	135 (62)	166 (23)
Non-Hispanic black	41 (27)	120 (35)	69 (32)	230 (33)
Hispanic	76 (50)	219 (65)	4 (2)	299 (42)
Other	3 (2)	0 (0)	9 (4)	12 (2)
Maternal education				
<High school	34 (23)	124 (37)	20 (9)	178 (25)
High school or GED	34 (23)	126 (37)	27 (12)	187 (26)
Some college	45 (30)	74 (22)	64 (29)	183 (26)
≥ College degree	38 (25)	15 (4)	106 (49)	159 (22)
Maternal work status during pregnancy (employed)	92 (61)	199 (59)	181 (83)	472 (67)
Parity (multiparous)	0 (0)	188 (55)	117 (54)	305 (43)
Maternal smoking during pregnancy (yes)	26 (17)	0 (0)	22 (10)	48 (7)
Maternal height (m) (mean ± SD)	64.0 (2.9)	63.7 (2.8)	65.0 (2.8)	64.2 (2.9)
Pre-pregnancy body mass index (kg/m ²)				
< 18.5	9 (6)	16 (5)	7 (4)	32 (5)
18.5-24.9	90 (60)	170 (51)	72 (43)	332 (51)
25-29.9	37 (25)	75 (23)	48 (29)	160 (25)
≥ 30	15 (10)	70 (21)	40 (24)	125 (19)
Missing	0	8	50	58
Gestational weight gain (lb)				
< 25	21 (16)	77 (25)	75 (45)	173 (29)
25-34.9	38 (29)	79 (26)	38 (23)	155 (26)
35-44.9	26 (20)	73 (24)	35 (21)	134 (22)
≥ 45	45 (35)	74 (24)	19 (11)	138 (23)
Missing	21	36	50	107
Year of urine collection				
1998-2000	133 (88)	94 (28)	0 (0)	227 (32)
2001-2003	18 (12)	125 (37)	26 (12)	169 (24)
2004-2006	0 (0)	120 (35)	191 (88)	311 (44)
Child's sex (male)	80 (53)	161 (47)	94 (43)	335 (47)

Characteristic	MSSM	CCEH	HOME	Pooled
Breastfed (ever)	94 (63)	256 (76)	176 (81)	526 (75)
Missing	1	4	1	6

Columbia Center for Children's Environmental Health (CCEH), Health Outcomes and Measures of the Environment Study (HOME), Mount Sinai School of Medicine Center for Children's Environmental Health (MSSM), standard deviation (SD)

Table 12. Characteristics of the birth cohorts at baseline by cohort and pooled

Characteristic	MSSM	CCEH	HOME	Pooled
Total (N)	376	407	360	1143
Maternal age at delivery (years)				
< 20	133 (35)	57 (14)	24 (7)	214 (19)
20–24	125 (33)	149 (37)	62 (17)	336 (29)
25–29	41 (11)	119 (29)	102 (28)	262 (23)
≥ 30	77 (20)	82 (20)	172 (48)	331 (29)
Race/ethnicity				
Non-Hispanic white	76 (20)	0 (0)	226 (63)	302 (26)
Non-Hispanic black	107 (28)	130 (32)	109 (30)	346 (30)
Hispanic	188 (50)	277 (68)	8 (2)	473 (41)
Other	5 (1)	0 (0)	15 (4)	20 (2)
Missing	0	0	2	2
Maternal education				
<High school	113 (30)	151 (37)	36 (10)	300 (26)
High school or GED	79 (21)	150 (37)	47 (13)	276 (24)
Some college	96 (26)	85 (21)	94 (26)	275 (24)
≥ College degree	88 (23)	20 (5)	181 (51)	289 (25)
Missing	0	1	2	3
Maternal work status during pregnancy (employed)	219 (58)	230 (57)	291 (81)	740 (65)
Missing	0	1	2	3
Parity (multiparous)	0 (0)	226 (56)	201 (56)	427 (37)
Missing	0	2	1	3
Maternal smoking during pregnancy (yes)	65 (17)	0 (0)	41 (11)	106 (9)
Maternal height (m) (mean ± SD)	64.0 (2.9)	63.7 (2.8)	64.9 (2.6)	64.2 (2.8)
Pre-pregnancy body mass index (kg/m ²)				
< 18.5	25 (7)	22 (6)	8 (3)	55 (5)
18.5-24.9	249 (66)	206 (52)	121 (48)	576 (56)
25-29.9	69 (18)	88 (22)	69 (27)	226 (22)
≥ 30	33 (9)	80 (20)	55 (22)	168 (16)
Missing	0	11	107	118
Gestational weight gain (lb)				
< 25	46 (14)	89 (25)	105 (42)	240 (25)
25-34.9	85 (26)	94 (26)	65 (26)	244 (26)
35-44.9	81 (24)	87 (24)	56 (22)	224 (24)
≥ 45	121 (36)	91 (25)	27 (11)	239 (25)
Missing	43	46	107	196
Year of urine collection				
1998-2000	339 (90)	105 (26)	0 (0)	444 (39)

Characteristic	MSSM	CCEH	HOME	Pooled
2001-2003	37 (10)	155 (38)	60 (17)	252 (22)
2004-2006	0 (0)	147 (36)	300 (83)	447 (39)
Child's sex (male)	207 (55)	197 (48)	172 (48)	576 (50)
Breastfed (ever)	191 (62)	293 (76)	281 (81)	765 (73)
Missing	68	22	11	101

Columbia Center for Children's Environmental Health (CCEH), Health Outcomes and Measures of the Environment Study (HOME), Mount Sinai School of Medicine Center for Children's Environmental Health (MSSM), standard deviation (SD)

Table 13. Distributions of phthalate metabolite concentrations in maternal urine samples during pregnancy, by cohort and pooled

Metabolite (µg/L)/ cohort	N	LOD	Percent detected	Geometric mean ^a	Minimum	25 th percentile	75 th percentile	Maximum
MEP								
MSSM	151	0.3	99	198.9	<LOD	81.0	495	7819
CCEH	339	0.5	100	162.0	7.79	69.4	334	6046
HOME	217	0.5	100	119.3	3.30	36.0	335	26004
Pooled	707		100	154.1	<LOD	63.4	353	26004
MnBP								
MSSM	151	0.4	100	32.0	0.800	14.3	75.2	4043
CCEH	339	0.6	100	38.0	1.20	20.3	81.3	1110
HOME	217	0.6	100	21.1	0.800	8.50	49.1	2240
Pooled	707		100	30.6	0.800	14.2	66.5	4043
MiBP								
MSSM	151	0.3	97	5.96	<LOD	2.90	15.3	76.6
CCEH	339	0.3	100	9.22	<LOD	5.10	19.0	374
HOME	217	0.3	95	3.90	<LOD	1.50	11.4	84.1
Pooled	707		98	6.45	<LOD	3.20	15.9	374
MCP								
MSSM	151	0.2	97	2.78	<LOD	1.50	5.50	129
CCEH	339	0.2	97	2.06	<LOD	1.20	4.10	32.7
HOME	217	0.2	98	1.73	<LOD	0.900	3.60	23.6
Pooled	707		97	2.08	<LOD	1.10	4.30	129
MBzP								
MSSM	151	0.1	99	13.3	<LOD	4.75	29.8	481
CCEH	339	0.2	100	13.5	<LOD	5.76	31.4	550
HOME	217	0.2	98	8.25	<LOD	3.74	22.5	653
Pooled	707		99	11.6	<LOD	4.75	28.7	653
ΣDEHP ^b								
MSSM	151	n/a	n/a	0.278	<LOD	0.129	0.507	19.9
CCEH	339	n/a	n/a	0.298	<LOD	0.141	0.608	18.6
HOME	217	n/a	n/a	0.248	<LOD	0.0959	0.537	6.56
Pooled	707	n/a	n/a	0.277	<LOD	0.128	0.562	19.9
MECP								
MSSM	151	0.3	99	35.2	<LOD	15.0	70.1	2055
CCEH	339	0.6	100	39.5	3.00	19.5	77.9	1840
HOME	217	0.6	100	30.7	1.70	12.6	63.6	641
Pooled	707		100	35.7	<LOD	16.1	74.4	2055
MEHP								
MSSM	151	0.3	99	20.6	<LOD	8.70	39.4	2051
CCEH	339	0.7	100	22.5	1.10	10.6	48.4	1750
HOME	217	0.7	100	20.4	<LOD	8.00	47.0	784
Pooled	707		100	21.4	<LOD	9.20	45.1	2051
MEHP								
MSSM	151	0.9	90	5.81	<LOD	3.00	11.8	478
CCEH	339	1.2	83	5.04	<LOD	2.20	12.8	613
HOME	217	1.2	77	4.23	<LOD	1.50	10.8	233
Pooled	707		83	4.92	<LOD	2.00	11.9	613
MEHP								
MSSM	151	0.5	99	18.3	<LOD	8.30	37.8	1335
CCEH	339	0.7	100	18.7	<LOD	8.90	37.6	1320
HOME	217	0.7	99	16.8	<LOD	6.40	36.9	448

Metabolite (µg/L)/ cohort	N	LOD	Percent detected	Geometric mean ^a	Minimum	25 th percentile	75 th percentile	Maximum
Pooled	707		99	18.0	<LOD	8.00	37.5	1335

Columbia Center for Children's Environmental Health (CCEH), Health Outcomes and Measures of the Environment Study (HOME), limit of detection (LOD), Mount Sinai School of Medicine Center for Children's Environmental Health (MSSM)

^a To compute the geometric mean, phthalate metabolite concentrations <LOD were replaced by LOD/ $\sqrt{2}$.

^b Micromoles per liter.

Table 14. Percent of children classified as overweight/obese by age at follow-up

Group/category	Age (years)			
	4 (<i>n</i> = 420)	5 (<i>n</i> = 361)	6 (<i>n</i> = 258)	7 (<i>n</i> = 377)
Overall	28	26	38	36
Cohort				
MSSM	35	30	28	36
CCEH	36	35	47	44
HOME	17	19	0	21
Child's sex				
Girls	28	27	39	35
Boys	27	25	37	37
Race/ethnicity				
Non-Hispanic white	17	16	13	15
Non-Hispanic black	22	30	37	32
Hispanic	43	34	44	48
Other	13	11	33	20

Columbia Center for Children's Environmental Health (CCEH), Health Outcomes and Measures of the Environment Study (HOME), Mount Sinai School of Medicine Center for Children's Environmental Health (MSSM)

Table 15. Age- and sex- standardized body mass index, weight, and height z-score distributions (mean \pm standard deviation) at follow-up by selected characteristics

Group/ category	<i>n</i>	Body mass index	Weight	Height
Overall	1416	0.47 \pm 1.20	0.59 \pm 1.16	0.54 \pm 1.12
Cohort				
MSSM	308	0.55 \pm 1.13	0.57 \pm 1.14	0.40 \pm 1.09
CCEH	632	0.64 \pm 1.32	0.92 \pm 1.11	0.86 \pm 1.04
HOME	476	0.19 \pm 1.00	0.18 \pm 1.11	0.22 \pm 1.16
Age (years)				
4	420	0.32 \pm 1.26	0.55 \pm 1.16	0.70 \pm 1.20
5	361	0.36 \pm 1.19	0.48 \pm 1.16	0.57 \pm 1.16
6	258	0.69 \pm 1.18	0.78 \pm 1.14	0.55 \pm 0.98
7	377	0.59 \pm 1.11	0.63 \pm 1.16	0.35 \pm 1.07
Child's sex				
Girls	756	0.44 \pm 1.20	0.54 \pm 1.17	0.53 \pm 1.15
Boys	660	0.50 \pm 1.20	0.65 \pm 1.14	0.57 \pm 1.10
Race/ethnicity				
Non-Hispanic white	358	0.17 \pm 0.93	0.18 \pm 1.02	0.23 \pm 1.12
Non-Hispanic black	463	0.46 \pm 1.21	0.60 \pm 1.20	0.58 \pm 1.19
Hispanic	570	0.69 \pm 1.29	0.88 \pm 1.12	0.73 \pm 1.02
Other	25	-0.09 \pm 1.23	-0.11 \pm 1.16	0.02 \pm 1.14

Columbia Center for Children's Environmental Health (CCEH), Health Outcomes and Measures of the Environment Study (HOME), Mount Sinai School of Medicine Center for Children's Environmental Health (MSSM), standard deviation (SD)

Table 16. Adjusted associations between prenatal urinary phthalate metabolite concentrations and overweight/obese status and body mass index (BMI) z-scores among children aged 4 to 7 years, overall and by child's sex

Metabolite / group ^a	Overweight/obese ^b	BMI z-score ^c
MEP		
Overall	0.68 (0.42, 1.08)	-0.05 (-0.15, 0.05)
Girls	0.60 (0.32, 1.12)	-0.14 (-0.28, 0.00)
Boys	0.85 (0.44, 1.65)	0.07 (-0.07, 0.21)
MnBP		
Overall	1.00 (0.51, 1.98)	0.03 (-0.12, 0.18)
Girls	1.29 (0.55, 3.01)	0.16 (-0.06, 0.39)
Boys	0.76 (0.30, 1.92)	-0.08 (-0.28, 0.13)
MiBP		
Overall	0.84 (0.44, 1.63)	0.01 (-0.14, 0.15)
Girls	0.92 (0.41, 2.01)	-0.07 (-0.26, 0.13)
Boys	0.67 (0.27, 1.64)	0.02 (-0.18, 0.22)
MCPP		
Overall	2.12 (1.16, 3.96)	-0.02 (-0.15, 0.11)
Girls	2.81 (1.35, 6.00)	0.06 (-0.11, 0.23)
Boys	1.54 (0.66, 3.66)	-0.09 (-0.28, 0.09)
MBzP		
Overall	0.63 (0.34, 1.15)	-0.07 (-0.20, 0.06)
Girls	0.66 (0.31, 1.37)	-0.09 (-0.27, 0.09)
Boys	0.69 (0.29, 1.60)	-0.04 (-0.22, 0.15)
ΣDEHP		
Overall	0.87 (0.53, 1.42)	-0.04 (-0.15, 0.06)
Girls	0.68 (0.36, 1.29)	-0.12 (-0.27, 0.02)
Boys	1.19 (0.59, 2.36)	0.04 (-0.11, 0.19)

Associations per standard deviation increase in natural log phthalate metabolite concentrations estimated in multiple metabolite models, adjusted for cohort, maternal race/ethnicity, maternal age at delivery, maternal education, maternal work status during pregnancy, maternal pre-pregnancy BMI, maternal height, gestational weight gain, maternal smoking during pregnancy, natural log creatinine, calendar date of urine collection, parity, child's sex, breastfeeding, and months of age at follow-up.

^a Number of children/number of follow-up visits for each group: 707/1416 (overall), 372/756 (girls), and 335/660 (boys).

^b Posterior mean of odds ratios (95% credible intervals).

^c Posterior mean of beta coefficients (95% credible intervals).

Table 17. Adjusted associations between prenatal urinary phthalate metabolite concentrations and overweight/obese status and body mass index (BMI) z-scores among children aged 4 to 7 years by race/ethnicity

Metabolite / group ^a	Overweight/obese ^b	BMI z-score ^c
MEP		
Non-Hispanic white	0.74 (0.27, 1.99)	0.01 (-0.19, 0.21)
Non-Hispanic black	0.69 (0.34, 1.37)	-0.05 (-0.21, 0.12)
Hispanic	0.62 (0.28, 1.32)	-0.08 (-0.25, 0.09)
MnBP		
Non-Hispanic white	1.12 (0.32, 3.90)	0.04 (-0.23, 0.32)
Non-Hispanic black	1.05 (0.43, 2.57)	0.00 (-0.27, 0.26)
Hispanic	0.85 (0.30, 2.47)	0.04 (-0.21, 0.29)
MiBP		
Non-Hispanic white	1.00 (0.32, 3.14)	-0.02 (-0.26, 0.22)
Non-Hispanic black	0.55 (0.22, 1.39)	-0.04 (-0.32, 0.25)
Hispanic	0.72 (0.27, 1.94)	0.01 (-0.22, 0.25)
MCPP		
Non-Hispanic white	1.17 (0.35, 3.87)	-0.13 (-0.39, 0.13)
Non-Hispanic black	1.79 (0.76, 4.22)	0.00 (-0.25, 0.24)
Hispanic	3.68 (1.55, 9.07)	0.08 (-0.11, 0.27)
MBzP		
Non-Hispanic white	0.74 (0.22, 2.50)	-0.16 (-0.42, 0.10)
Non-Hispanic black	0.58 (0.25, 1.37)	-0.08 (-0.32, 0.16)
Hispanic	0.63 (0.26, 1.51)	-0.01 (-0.21, 0.18)
ΣDEHP		
Non-Hispanic white	1.44 (0.58, 3.60)	0.10 (-0.09, 0.29)
Non-Hispanic black	0.98 (0.46, 2.10)	-0.04 (-0.25, 0.16)
Hispanic	0.59 (0.27, 1.27)	-0.14 (-0.31, 0.03)

Associations per standard deviation increase in natural log phthalate metabolite concentrations estimated in multiple metabolite models, adjusted for cohort, maternal race/ethnicity, maternal age at delivery, maternal education, maternal work status during pregnancy, maternal pre-pregnancy BMI, maternal height, gestational weight gain, maternal smoking during pregnancy, natural log creatinine, calendar date of urine collection, parity, child's sex, breastfeeding, and months of age at follow-up.

^a Number of children/number of follow-up visits for each group: 166/358 (non-Hispanic white), 230/463 (non-Hispanic black), and 299/570 (Hispanic).

^b Posterior mean of odds ratios (95% credible intervals).

^c Posterior mean of beta coefficients (95% credible intervals).

Table 18. Adjusted associations between prenatal urinary phthalate metabolite concentrations and weight and height z-scores among children aged 4 to 7 years, overall and by child's sex

Metabolite / group ^a	Weight z-score ^b	Height z-score ^b
MEP		
Overall	-0.08 (-0.18, 0.01)	-0.07 (-0.15, 0.02)
Girls	-0.18 (-0.31, -0.05)	-0.14 (-0.26, -0.01)
Boys	0.04 (-0.09, 0.17)	0.02 (-0.10, 0.14)
MnBP		
Overall	0.03 (-0.11, 0.18)	0.02 (-0.11, 0.15)
Girls	0.14 (-0.07, 0.34)	0.03 (-0.16, 0.23)
Boys	-0.05 (-0.24, 0.14)	0.00 (-0.18, 0.18)
MiBP		
Overall	0.03 (-0.11, 0.16)	0.05 (-0.08, 0.18)
Girls	0.01 (-0.17, 0.19)	0.09 (-0.08, 0.26)
Boys	0.00 (-0.19, 0.18)	0.01 (-0.16, 0.18)
MCPP		
Overall	0.00 (-0.12, 0.12)	-0.02 (-0.13, 0.10)
Girls	0.08 (-0.08, 0.24)	0.03 (-0.13, 0.18)
Boys	-0.09 (-0.27, 0.08)	-0.07 (-0.24, 0.09)
MBzP		
Overall	-0.09 (-0.22, 0.03)	-0.10 (-0.21, 0.02)
Girls	-0.11 (-0.27, 0.06)	-0.07 (-0.23, 0.08)
Boys	-0.05 (-0.22, 0.12)	-0.10 (-0.26, 0.06)
ΣDEHP		
Overall	-0.05 (-0.15, 0.05)	-0.03 (-0.12, 0.06)
Girls	-0.07 (-0.20, 0.06)	0.02 (-0.10, 0.15)
Boys	-0.03 (-0.17, 0.11)	-0.09 (-0.22, 0.04)

Associations per standard deviation increase in natural log phthalate metabolite concentrations estimated in multiple metabolite models, adjusted for cohort, maternal race/ethnicity, maternal age at delivery, maternal education, maternal work status during pregnancy, maternal pre-pregnancy body mass index, maternal height, gestational weight gain, maternal smoking during pregnancy, natural log creatinine, calendar date of urine collection, parity, child's sex, breastfeeding, and months of age at follow-up.

^a Number of children/number of follow-up visits for each group: 707/1416 (overall), 372/756 (girls), and 335/660 (boys).

^b Posterior mean of beta coefficients (95% credible intervals).

Table 19. Adjusted associations between prenatal urinary phthalate metabolite concentrations and weight and height z-scores among children aged 4 to 7 years by race/ethnicity

Metabolite / group ^a	Weight z-score ^b	Height z-score ^b
MEP		
Non-Hispanic white	-0.08 (-0.26, 0.11)	-0.14 (-0.32, 0.03)
Non-Hispanic black	-0.05 (-0.20, 0.11)	0.02 (-0.12, 0.17)
Hispanic	-0.13 (-0.29, 0.03)	-0.11 (-0.26, 0.03)
MnBP		
Non-Hispanic white	0.01 (-0.25, 0.27)	-0.03 (-0.27, 0.21)
Non-Hispanic black	0.03 (-0.22, 0.28)	0.06 (-0.17, 0.29)
Hispanic	0.06 (-0.16, 0.30)	0.04 (-0.17, 0.26)
MiBP		
Non-Hispanic white	0.08 (-0.15, 0.30)	0.15 (-0.06, 0.36)
Non-Hispanic black	0.05 (-0.21, 0.32)	0.14 (-0.10, 0.39)
Hispanic	-0.05 (-0.27, 0.16)	-0.08 (-0.28, 0.12)
MCP		
Non-Hispanic white	-0.11 (-0.36, 0.13)	-0.06 (-0.29, 0.16)
Non-Hispanic black	0.10 (-0.13, 0.32)	0.14 (-0.07, 0.36)
Hispanic	0.05 (-0.12, 0.22)	-0.05 (-0.21, 0.12)
MBzP		
Non-Hispanic white	-0.10 (-0.35, 0.14)	-0.03 (-0.26, 0.20)
Non-Hispanic black	-0.24 (-0.46, -0.02)	-0.35 (-0.56, -0.14)
Hispanic	0.01 (-0.17, 0.19)	0.04 (-0.13, 0.20)
ΣDEHP		
Non-Hispanic white	0.06 (-0.11, 0.24)	0.02 (-0.14, 0.18)
Non-Hispanic black	-0.12 (-0.31, 0.06)	-0.18 (-0.35, 0.00)
Hispanic	-0.09 (-0.24, 0.07)	0.01 (-0.13, 0.16)

Associations per standard deviation increase in natural log phthalate metabolite concentrations estimated in multiple metabolite models, adjusted for cohort, maternal race/ethnicity, maternal age at delivery, maternal education, maternal work status during pregnancy, maternal pre-pregnancy body mass index, maternal height, gestational weight gain, maternal smoking during pregnancy, natural log creatinine, calendar date of urine collection, parity, child's sex, breastfeeding, and months of age at follow-up.

^a Number of children/number of follow-up visits for each group: 166/358 (non-Hispanic white), 230/463 (non-Hispanic black), and 299/570 (Hispanic).

^b Posterior mean of beta coefficients (95% credible intervals).

Table 20. Adjusted associations between prenatal urinary phthalate metabolite concentrations and overweight/obese status by cohort, child's sex, and race/ethnicity

Metabolite /cohort	Overall	Girls	Boys	Non-Hispanic white	Non-Hispanic black	Hispanic
MEP						
MSSM	0.89 (0.40, 1.98)	0.79 (0.32, 1.94)	1.39 (0.46, 4.15)	0.97 (0.21, 4.36)	0.82 (0.32, 2.12)	0.86 (0.25, 2.88)
CCEH	0.59 (0.27, 1.26)	0.58 (0.21, 1.56)	0.61 (0.21, 1.73)		0.73 (0.22, 2.36)	0.50 (0.18, 1.30)
HOME	0.56 (0.25, 1.24)	0.51 (0.19, 1.31)	0.66 (0.20, 2.10)	0.64 (0.20, 2.05)	0.50 (0.16, 1.50)	
MnBP						
MSSM	0.69 (0.27, 1.80)	0.96 (0.32, 2.85)	0.46 (0.13, 1.60)	0.63 (0.12, 3.17)	0.67 (0.22, 2.05)	0.52 (0.12, 2.25)
CCEH	1.18 (0.42, 3.35)	1.61 (0.46, 5.64)	0.92 (0.22, 3.77)		1.23 (0.30, 5.05)	1.16 (0.30, 4.39)
HOME	1.49 (0.48, 4.60)	1.60 (0.45, 5.78)	1.55 (0.28, 8.11)	1.27 (0.24, 6.42)	1.59 (0.38, 6.61)	
MiBP						
MSSM	0.87 (0.34, 2.22)	0.99 (0.36, 2.78)	0.68 (0.18, 2.49)	0.77 (0.16, 3.72)	0.55 (0.18, 1.72)	0.85 (0.22, 3.34)
CCEH	0.66 (0.24, 1.79)	0.79 (0.24, 2.56)	0.37 (0.08, 1.57)		0.54 (0.13, 2.14)	0.60 (0.17, 2.08)
HOME	1.03 (0.36, 3.04)	1.00 (0.29, 3.57)	0.94 (0.21, 4.35)	1.16 (0.29, 4.94)	0.54 (0.11, 2.55)	
MCPP						
MSSM	2.15 (0.88, 5.31)	2.94 (1.09, 8.04)	1.82 (0.53, 6.23)	1.47 (0.32, 6.68)	1.89 (0.64, 5.55)	3.84 (1.00, 14.76)
CCEH	2.64 (1.14, 6.41)	3.50 (1.30, 9.96)	1.93 (0.56, 6.90)		1.79 (0.53, 6.27)	3.74 (1.28, 11.58)
HOME	1.34 (0.41, 4.45)	1.95 (0.50, 7.70)	0.79 (0.16, 4.05)	0.95 (0.19, 4.77)	1.86 (0.41, 8.63)	
MBzP						
MSSM	0.79 (0.31, 1.99)	0.80 (0.29, 2.20)	0.85 (0.22, 3.15)	0.75 (0.15, 3.81)	0.69 (0.23, 2.05)	0.96 (0.23, 3.92)
CCEH	0.46 (0.20, 1.03)	0.59 (0.21, 1.62)	0.42 (0.13, 1.31)		0.39 (0.11, 1.30)	0.49 (0.17, 1.37)
HOME	0.78 (0.25, 2.42)	0.66 (0.19, 2.31)	1.13 (0.22, 5.67)	0.80 (0.17, 3.63)	1.02 (0.22, 4.62)	
ΣDEHP						
MSSM	0.71 (0.33, 1.51)	0.59 (0.24, 1.46)	0.81 (0.30, 2.17)	1.32 (0.34, 5.10)	0.76 (0.28, 2.07)	0.44 (0.14, 1.36)
CCEH	0.90 (0.42, 1.92)	0.67 (0.26, 1.67)	1.47 (0.46, 4.67)		1.11 (0.37, 3.29)	0.71 (0.26, 1.92)
HOME	1.13 (0.44, 2.85)	0.76 (0.25, 2.28)	2.06 (0.55, 7.53)	1.53 (0.48, 4.91)	0.96 (0.24, 3.83)	

Columbia Center for Children's Environmental Health (CCEH), Health Outcomes and Measures of the Environment Study (HOME), Mount Sinai School of Medicine Center for Children's Environmental Health (MSSM)

Posterior mean of odds ratios (95% credible intervals) per standard deviation increase in natural log phthalate metabolite concentrations estimated in multiple metabolite models, adjusted for cohort, maternal race/ethnicity, maternal age at delivery, maternal education, maternal work status during pregnancy, maternal pre-pregnancy body mass index, maternal height, gestational weight gain, maternal smoking during pregnancy, natural log creatinine, calendar date of urine collection, parity, child's sex, breastfeeding, and months of age at follow-up.

Table 21. Adjusted associations between prenatal urinary phthalate metabolite concentrations and body mass index z-scores by cohort, child's sex, and race/ethnicity

	Metabolite /cohort	Overall					Non-Hispanic		Hispanic
		Girls		Boys		white		black	
88	MEP								
	MSSM	0.14 (-0.09, 0.37)	0.03 (-0.28, 0.34)	0.29 (-0.03, 0.61)	0.06 (-0.44, 0.56)	0.08 (-0.26, 0.43)	0.15 (-0.19, 0.49)		
	CCEH	-0.10 (-0.27, 0.07)	-0.14 (-0.37, 0.09)	-0.02 (-0.25, 0.21)		0.03 (-0.26, 0.31)	-0.16 (-0.36, 0.05)		
	HOME	-0.06 (-0.22, 0.09)	-0.20 (-0.41, 0.00)	0.11 (-0.12, 0.34)	0.01 (-0.21, 0.23)	-0.11 (-0.36, 0.14)			
	MnBP								
	MSSM	-0.23 (-0.54, 0.09)	-0.04 (-0.52, 0.45)	-0.27 (-0.64, 0.10)	-0.16 (-0.73, 0.41)	-0.27 (-0.74, 0.21)	-0.29 (-0.77, 0.19)		
	CCEH	0.04 (-0.21, 0.28)	0.27 (-0.07, 0.61)	-0.17 (-0.51, 0.17)		-0.17 (-0.61, 0.27)	0.15 (-0.15, 0.44)		
	HOME	0.18 (-0.07, 0.44)	0.18 (-0.16, 0.52)	0.19 (-0.16, 0.55)	0.08 (-0.24, 0.40)	0.21 (-0.22, 0.64)			
	MiBP								
	MSSM	-0.02 (-0.30, 0.27)	0.04 (-0.33, 0.40)	-0.14 (-0.54, 0.25)	-0.39 (-0.95, 0.17)	-0.16 (-0.69, 0.38)	0.03 (-0.37, 0.42)		
	CCEH	0.05 (-0.19, 0.29)	-0.08 (-0.40, 0.23)	0.07 (-0.27, 0.41)		0.21 (-0.22, 0.63)	-0.01 (-0.29, 0.28)		
	HOME	-0.02 (-0.24, 0.21)	-0.11 (-0.41, 0.20)	0.03 (-0.27, 0.34)	0.05 (-0.22, 0.32)	-0.19 (-0.67, 0.28)			
	MCP								
	MSSM	0.17 (-0.12, 0.46)	0.19 (-0.17, 0.55)	0.15 (-0.25, 0.55)	-0.05 (-0.54, 0.44)	0.19 (-0.30, 0.67)	0.40 (-0.03, 0.83)		
	CCEH	-0.05 (-0.23, 0.13)	0.06 (-0.17, 0.29)	-0.17 (-0.45, 0.12)		-0.09 (-0.42, 0.23)	-0.03 (-0.25, 0.18)		
	HOME	-0.05 (-0.31, 0.21)	-0.03 (-0.41, 0.34)	-0.07 (-0.41, 0.28)	-0.10 (-0.42, 0.22)	0.21 (-0.27, 0.70)			
	MBzP								
	MSSM	-0.03 (-0.34, 0.27)	-0.02 (-0.39, 0.35)	-0.12 (-0.59, 0.35)	-0.03 (-0.63, 0.57)	0.07 (-0.42, 0.56)	-0.01 (-0.45, 0.42)		
	CCEH	-0.09 (-0.27, 0.09)	-0.08 (-0.33, 0.17)	-0.03 (-0.28, 0.23)		-0.12 (-0.44, 0.2)	-0.07 (-0.30, 0.15)		
	HOME	-0.13 (-0.38, 0.11)	-0.12 (-0.44, 0.19)	-0.15 (-0.51, 0.21)	-0.18 (-0.47, 0.12)	-0.03 (-0.50, 0.43)			
	ΣDEHP								
	MSSM	-0.13 (-0.32, 0.07)	-0.16 (-0.46, 0.14)	-0.06 (-0.32, 0.20)	0.28 (-0.12, 0.68)	-0.20 (-0.60, 0.20)	-0.28 (-0.56, 0.00)		
	CCEH	-0.05 (-0.21, 0.12)	-0.19 (-0.40, 0.03)	0.12 (-0.15, 0.38)		-0.06 (-0.34, 0.21)	-0.05 (-0.26, 0.17)		
	HOME	0.05 (-0.14, 0.24)	0.03 (-0.23, 0.28)	0.10 (-0.18, 0.37)	0.07 (-0.15, 0.30)	-0.02 (-0.44, 0.40)			

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Posterior mean of beta coefficients (95% credible intervals) per standard deviation increase in natural log phthalate metabolite concentrations estimated in multiple metabolite models, adjusted for cohort, maternal race/ethnicity, maternal age at delivery, maternal education, maternal work status during pregnancy, maternal pre-pregnancy body mass index, maternal height, gestational weight gain, maternal smoking during pregnancy, natural log creatinine, calendar date of urine collection, parity, child's sex, breastfeeding, and months of age at follow-up.

Table 22. Adjusted associations between prenatal urinary phthalate metabolite concentrations and weight z-scores by cohort, child's sex, and race/ethnicity

Metabolite /cohort	Overall	Girls	Boys	Non-Hispanic white	Non-Hispanic black	Hispanic
MEP						
MSSM	0.15 (-0.06, 0.36)	0.05 (-0.24, 0.33)	0.33 (0.04, 0.63)	0.22 (-0.11, 0.55)	-0.02 (-0.48, 0.44)	0.12 (-0.19, 0.43)
CCEH	-0.16 (-0.32, -0.01)	-0.20 (-0.41, 0.02)	-0.09 (-0.30, 0.12)	-0.02 (-0.29, 0.24)		-0.21 (-0.4, -0.02)
HOME	-0.09 (-0.24, 0.05)	-0.25 (-0.45, -0.06)	0.11 (-0.10, 0.32)	-0.15 (-0.38, 0.08)	-0.08 (-0.28, 0.12)	
MnBP						
MSSM	-0.30 (-0.59, 0.00)	-0.13 (-0.58, 0.34)	-0.35 (-0.69, -0.01)	-0.37 (-0.82, 0.07)	-0.27 (-0.80, 0.25)	-0.26 (-0.71, 0.18)
CCEH	0.10 (-0.12, 0.33)	0.31 (0.00, 0.62)	-0.09 (-0.41, 0.22)	-0.02 (-0.43, 0.39)		0.17 (-0.11, 0.44)
HOME	0.20 (-0.04, 0.43)	0.10 (-0.21, 0.41)	0.32 (-0.01, 0.66)	0.27 (-0.14, 0.67)	0.07 (-0.23, 0.36)	
MiBP						
MSSM	0.03 (-0.24, 0.29)	0.11 (-0.23, 0.45)	-0.13 (-0.49, 0.23)	-0.10 (-0.62, 0.41)	-0.23 (-0.74, 0.30)	0.03 (-0.34, 0.39)
CCEH	-0.04 (-0.26, 0.18)	-0.10 (-0.39, 0.20)	-0.06 (-0.37, 0.25)	0.18 (-0.22, 0.57)		-0.11 (-0.37, 0.15)
HOME	0.08 (-0.12, 0.29)	0.03 (-0.25, 0.32)	0.08 (-0.20, 0.36)	0.05 (-0.39, 0.49)	0.13 (-0.12, 0.38)	
MCP						
MSSM	0.20 (-0.08, 0.46)	0.23 (-0.11, 0.57)	0.22 (-0.15, 0.59)	0.32 (-0.14, 0.77)	0.04 (-0.42, 0.50)	0.34 (-0.05, 0.74)
CCEH	-0.04 (-0.20, 0.13)	0.04 (-0.17, 0.25)	-0.12 (-0.38, 0.14)	-0.02 (-0.31, 0.28)		-0.03 (-0.23, 0.17)
HOME	0.00 (-0.24, 0.24)	0.09 (-0.26, 0.44)	-0.12 (-0.44, 0.19)	0.37 (-0.08, 0.82)	-0.11 (-0.40, 0.19)	
MBzP						
MSSM	-0.11 (-0.39, 0.17)	-0.03 (-0.37, 0.31)	-0.30 (-0.74, 0.14)	-0.13 (-0.60, 0.34)	-0.02 (-0.58, 0.53)	-0.07 (-0.48, 0.33)
CCEH	-0.09 (-0.25, 0.08)	-0.10 (-0.33, 0.13)	0.00 (-0.24, 0.23)	-0.25 (-0.55, 0.05)		-0.02 (-0.22, 0.18)
HOME	-0.18 (-0.40, 0.05)	-0.13 (-0.42, 0.16)	-0.24 (-0.57, 0.10)	-0.25 (-0.68, 0.19)	-0.11 (-0.38, 0.16)	
Σ DEHP						
MSSM	-0.09 (-0.27, 0.09)	-0.07 (-0.35, 0.20)	-0.04 (-0.28, 0.20)	-0.29 (-0.67, 0.08)	0.19 (-0.17, 0.56)	-0.17 (-0.42, 0.09)
CCEH	-0.04 (-0.19, 0.12)	-0.11 (-0.31, 0.09)	0.06 (-0.19, 0.30)	-0.11 (-0.36, 0.14)		-0.03 (-0.23, 0.17)
HOME	-0.01 (-0.18, 0.17)	0.00 (-0.23, 0.24)	-0.02 (-0.27, 0.23)	-0.18 (-0.58, 0.21)	0.06 (-0.15, 0.26)	

Columbia Center for Children's Environmental Health (CCEH), Health Outcomes and Measures of the Environment Study (HOME), Mount Sinai School of Medicine Center for Children's Environmental Health (MSSM)

Posterior mean of beta coefficients (95% credible intervals) per standard deviation increase in natural log phthalate metabolite concentrations estimated in multiple metabolite models, adjusted for cohort, maternal race/ethnicity, maternal age at delivery, maternal education, maternal work status during pregnancy, maternal pre-pregnancy body mass index, maternal height, gestational weight gain, maternal smoking during pregnancy, natural log creatinine, calendar date of urine collection, parity, child's sex, breastfeeding, and months of age at follow-up.

Table 23. Adjusted associations between prenatal urinary phthalate metabolite concentrations and height z-scores by cohort, child's sex, and race/ethnicity

Metabolite /cohort	Overall	Girls	Boys	Non-Hispanic white	Non-Hispanic black	Hispanic
MEP						
MSSM	0.12 (-0.08, 0.31)	0.09 (-0.18, 0.37)	0.25 (-0.03, 0.53)	-0.09 (-0.52, 0.35)	0.30 (-0.01, 0.62)	0.05 (-0.24, 0.35)
CCEH	-0.14 (-0.28, 0.01)	-0.16 (-0.36, 0.04)	-0.09 (-0.29, 0.11)		-0.02 (-0.27, 0.23)	-0.16 (-0.34, 0.01)
HOME	-0.06 (-0.20, 0.07)	-0.20 (-0.38, -0.02)	0.10 (-0.10, 0.29)	-0.14 (-0.33, 0.05)	-0.06 (-0.28, 0.15)	
MnBP						
MSSM	-0.22 (-0.50, 0.05)	-0.18 (-0.61, 0.26)	-0.24 (-0.57, 0.08)	-0.24 (-0.74, 0.26)	-0.29 (-0.72, 0.14)	-0.11 (-0.53, 0.31)
CCEH	0.12 (-0.09, 0.33)	0.19 (-0.11, 0.49)	0.01 (-0.29, 0.30)		0.16 (-0.22, 0.54)	0.09 (-0.16, 0.34)
HOME	0.11 (-0.11, 0.34)	-0.04 (-0.33, 0.26)	0.30 (-0.01, 0.62)	0.02 (-0.26, 0.29)	0.20 (-0.18, 0.58)	
MiBP						
MSSM	0.08 (-0.17, 0.33)	0.15 (-0.18, 0.48)	-0.04 (-0.38, 0.31)	0.09 (-0.40, 0.59)	0.03 (-0.47, 0.53)	0.04 (-0.30, 0.39)
CCEH	-0.10 (-0.31, 0.11)	-0.05 (-0.33, 0.23)	-0.12 (-0.42, 0.18)		0.12 (-0.25, 0.49)	-0.15 (-0.40, 0.09)
HOME	0.16 (-0.03, 0.36)	0.16 (-0.11, 0.43)	0.13 (-0.14, 0.40)	0.16 (-0.07, 0.39)	0.24 (-0.18, 0.65)	
MCP						
MSSM	0.11 (-0.14, 0.37)	0.16 (-0.16, 0.48)	0.18 (-0.17, 0.53)	0.13 (-0.30, 0.57)	0.32 (-0.12, 0.76)	0.07 (-0.30, 0.45)
CCEH	-0.04 (-0.19, 0.12)	-0.04 (-0.24, 0.16)	-0.02 (-0.27, 0.23)		0.07 (-0.22, 0.34)	-0.05 (-0.24, 0.13)
HOME	0.03 (-0.20, 0.26)	0.16 (-0.17, 0.48)	-0.14 (-0.45, 0.16)	-0.09 (-0.36, 0.19)	0.34 (-0.08, 0.76)	
MBzP						
MSSM	-0.21 (-0.48, 0.06)	-0.05 (-0.38, 0.28)	-0.48 (-0.89, -0.06)	-0.13 (-0.66, 0.39)	-0.38 (-0.83, 0.08)	-0.13 (-0.51, 0.25)
CCEH	-0.05 (-0.20, 0.11)	-0.06 (-0.28, 0.16)	-0.01 (-0.23, 0.21)		-0.32 (-0.60, -0.04)	0.06 (-0.13, 0.26)
HOME	-0.17 (-0.38, 0.04)	-0.09 (-0.36, 0.19)	-0.27 (-0.58, 0.04)	-0.01 (-0.26, 0.24)	-0.40 (-0.81, 0.00)	
Σ DEHP						
MSSM	-0.01 (-0.18, 0.16)	0.05 (-0.22, 0.31)	0.02 (-0.20, 0.24)	0.00 (-0.34, 0.35)	-0.29 (-0.65, 0.07)	0.05 (-0.20, 0.29)
CCEH	-0.03 (-0.17, 0.12)	0.02 (-0.17, 0.20)	-0.07 (-0.30, 0.16)		-0.14 (-0.38, 0.09)	0.00 (-0.19, 0.18)
HOME	-0.04 (-0.21, 0.12)	0.00 (-0.23, 0.22)	-0.10 (-0.34, 0.14)	0.04 (-0.15, 0.24)	-0.25 (-0.62, 0.13)	

Columbia Center for Children's Environmental Health (CCEH), Health Outcomes and Measures of the Environment Study (HOME), Mount Sinai School of Medicine Center for Children's Environmental Health (MSSM)

Posterior mean of beta coefficients (95% credible intervals) per standard deviation increase in natural log phthalate metabolite concentrations estimated in multiple metabolite models, adjusted for cohort, maternal race/ethnicity, maternal age at delivery, maternal education, maternal work status during pregnancy, maternal pre-pregnancy body mass index, maternal height, gestational weight gain, maternal smoking during pregnancy, natural log creatinine, calendar date of urine collection, parity, child's sex, breastfeeding, and months of age at follow-up.

Table 24. Sensitivity analyses for confounding among metabolites and loss to follow-up

Metabolite/outcome	Multiple metabolite models ^a (N = 707)	Single metabolite models ^b (N = 707)	Multiple metabolite models accounting for loss to follow-up ^c (N = 1143)
MEP			
Overweight/obese ^d	0.68 (0.42, 1.08)	0.66 (0.42, 1.03)	0.69 (0.43, 1.12)
BMI z-score ^e	-0.05 (-0.15, 0.05)	-0.06 (-0.15, 0.03)	-0.04 (-0.14, 0.06)
Weight z-score ^e	-0.08 (-0.18, 0.01)	-0.10 (-0.19, -0.01)	-0.10 (-0.19, -0.01)
Height z-score ^e	-0.07 (-0.15, 0.02)	-0.08 (-0.16, 0.00)	-0.08 (-0.16, 0.01)
MnBP			
Overweight/obese ^d	1.00 (0.51, 1.98)	0.91 (0.56, 1.50)	0.96 (0.49, 1.91)
BMI z-score ^e	0.03 (-0.12, 0.18)	-0.03 (-0.14, 0.08)	-0.01 (-0.16, 0.14)
Weight z-score ^e	0.03 (-0.11, 0.18)	-0.04 (-0.14, 0.06)	0.04 (-0.10, 0.18)
Height z-score ^e	0.02 (-0.11, 0.15)	-0.04 (-0.14, 0.05)	0.03 (-0.11, 0.16)
MiBP			
Overweight/obese ^d	0.84 (0.44, 1.63)	0.78 (0.46, 1.31)	0.87 (0.45, 1.67)
BMI z-score ^e	0.01 (-0.14, 0.15)	-0.04 (-0.15, 0.08)	0.04 (-0.11, 0.18)
Weight z-score ^e	0.03 (-0.11, 0.16)	-0.04 (-0.15, 0.07)	0.06 (-0.08, 0.19)
Height z-score ^e	0.05 (-0.08, 0.18)	-0.02 (-0.12, 0.08)	0.07 (-0.06, 0.20)
MCP			
Overweight/obese ^d	2.12 (1.16, 3.96)	1.42 (0.88, 2.30)	2.22 (1.21, 4.12)
BMI z-score ^e	-0.02 (-0.15, 0.11)	-0.05 (-0.16, 0.05)	0.03 (-0.11, 0.16)
Weight z-score ^e	0.00 (-0.12, 0.12)	-0.05 (-0.15, 0.05)	-0.01 (-0.13, 0.12)
Height z-score ^e	-0.02 (-0.13, 0.10)	-0.06 (-0.15, 0.04)	-0.03 (-0.14, 0.09)
MBzP			
Overweight/obese ^d	0.63 (0.34, 1.15)	0.71 (0.44, 1.15)	0.62 (0.34, 1.13)
BMI z-score ^e	-0.07 (-0.20, 0.06)	-0.08 (-0.19, 0.02)	-0.06 (-0.19, 0.08)
Weight z-score ^e	-0.09 (-0.22, 0.03)	-0.10 (-0.20, 0.00)	-0.12 (-0.24, 0.00)
Height z-score ^e	-0.10 (-0.21, 0.02)	-0.10 (-0.20, -0.01)	-0.11 (-0.22, 0.00)

Metabolite/outcome	Multiple metabolite models ^a (N = 707)	Single metabolite models ^b (N = 707)	Multiple metabolite models accounting for loss to follow-up ^c (N = 1143)
ΣDEHP			
Overweight/obese ^d	0.87 (0.53, 1.42)	0.89 (0.57, 1.38)	0.86 (0.53, 1.41)
BMI z-score ^e	-0.04 (-0.15, 0.06)	-0.07 (-0.16, 0.03)	-0.04 (-0.15, 0.06)
Weight z-score ^e	-0.05 (-0.15, 0.05)	-0.08 (-0.17, 0.01)	-0.03 (-0.13, 0.06)
Height z-score ^e	-0.03 (-0.12, 0.06)	-0.06 (-0.14, 0.02)	-0.02 (-0.11, 0.07)

BMI Body mass index

Associations per standard deviation increase in natural log phthalate metabolite concentrations, adjusted for cohort, maternal race/ethnicity, maternal age at delivery, maternal education, maternal work status during pregnancy, maternal pre-pregnancy BMI, maternal height, gestational weight gain, maternal smoking during pregnancy, natural log creatinine, calendar date of urine collection, parity, child's sex, breastfeeding, and months of age at follow-up.

^a Associations among children with at least one follow-up visit estimated in a multiple metabolite linear mixed effects regression model.

^b Associations among children with at least one follow-up visit estimated in a separate linear mixed effects regression model for each metabolite.

^c Associations among all children with measured prenatal phthalate metabolite concentrations estimated in a multiple metabolite linear mixed effects regression model using a selection model for nonignorable missing outcome data.

^d Posterior mean of odds ratios (95% credible intervals).

^e Posterior mean of beta coefficients (95% credible intervals).

CHAPTER VI. CONCLUSIONS

A. Summary of Findings

A growing body of literature suggests that prenatal environmental chemical exposures may be a factor in the rise in childhood obesity rates in the last several decades. In this dissertation, we examined whether prenatal phthalate exposures are associated with body size in early childhood.

In the first Specific Aim, we examined whether third trimester maternal urinary concentrations of phthalate metabolites were associated with percent fat mass, measured using bio-electrical impedance analysis, among children aged 4 to 9 years. We found that children in the highest tertile of creatinine-corrected Σ DEHP metabolite concentrations had lower percent fat mass than children in the lowest tertile of Σ DEHP metabolite concentrations ($\beta = -3.06$, 95% CI: -5.99, -0.09). There was no evidence of associations between other phthalate metabolites and percent body fat. Furthermore, we did not observe heterogeneity of associations by child sex, though our sample size was limited.

In the second Specific Aim, we assessed prenatal phthalate exposures in relation to several anthropometry-based outcomes among children aged 4 to 7 years in a pooled sample comprised of children in three birth cohorts. We found evidence that higher prenatal exposure to MCP, a high molecular weight phthalate found in food packaging and plastics, was associated with higher odds of overweight or obese status at follow-up (OR per SD = 2.12, 95% CI = 1.16, 3.96). We also observed an inverse association of maternal urinary MEP concentrations with BMI, weight, and height z-scores among girls, but not boys. MBzP was negatively associated with weight and height z-scores among non-Hispanic black children only.

We did not observe associations between MnBP, MiBP, or Σ DEHP and measures of childhood body size.

Taken together, these results do not provide strong evidence for the hypothesis that prenatal phthalate exposures are obesogenic. While we observed a positive association between MCPP and overweight/obese status in Aim 2, there was only weak evidence of an association of MCPP with percent fat mass in Aim 1. MCPP was not associated with percent fat mass in models assessing continuous MCPP concentrations (β per SD = 0.63, 95% CI: -1.55, 2.82). Comparing the highest to lowest tertile of MCPP exposure yielded positive associations with percent fat mass (β = 1.79, 95% CI: -1.61, 5.17) and BMI z-scores (β = 0.32, 95% CI = -0.16, 0.79), though the CIs were wide and crossed the null.

Our finding of increased odds of overweight/obesity in Aim 2 may be evidence that MCPP is a weak obesogen. Consistent with our findings, a study of first trimester urinary phthalate metabolite concentrations and cord blood levels of two adipocyte-produced hormones reported associations of MCPP, but not other phthalate metabolites, with leptin and adiponectin levels (Ashley-Martin et al. 2014). Male infants in the second, third, and fourth quartiles of MCPP exposure had increased odds of high ($\geq 90^{\text{th}}$ percentile) leptin and females in the highest quartile of MCPP exposure had higher odds of high ($\geq 90^{\text{th}}$ percentile) adiponectin.

It is possible that we could not fully control for confounding by shared maternal and child diet as MCPP exposure is most commonly through packaging of high fat foods. However, the inverse association of Σ DEHP concentrations with overweight/obese status provides indirect evidence that associations are not strongly confounded by diet since Σ DEHP and MCPP exposures are associated with intake of similar foods (Serrano et al. 2014).

We observed stronger associations of MCPP with overweight/obese status among girls than boys. It is not immediately clear whether this is true biological variability in this association as MCPP has not been shown to affect estrogen (Chen et al. 2014) or androgen (Saillenfait et al. 2011) activity in animal studies. However, this phthalate is not well-studied in the

toxicological literature. There is some evidence that MCPP may impact the thyroid and liver, which are additional potential pathways for an effect of this phthalate on obesity (Poon et al. 1997).

MCPP was also more strongly associated with overweight and obesity among Hispanic children than among non-Hispanic children. While interpreting heterogeneity of associations by race/ethnicity in Aim 2 is somewhat complicated by other differences between the three cohorts, differences in associations by race/ethnicity have been observed in previous studies of phthalates in relation to body size. For example, a cross-sectional study of urinary phthalate metabolites measured in a sample of NHANES children and adolescents reported an association of low molecular weight phthalates with higher odds of overweight and obesity among non-Hispanic black children, but not non-Hispanic white or Hispanic children (Trasande et al. 2013). The underlying mechanism for such a difference is unclear. A causal association could be due to differences in genetic susceptibility or in the childhood food environment. For example, if MCPP affects satiety, ethnic differences related to food availability or nutrient content may explain differences in associations by ethnicity. Hispanic children had a higher prevalence of overweight/obesity and greater BMI z-scores than non-Hispanic white and black children. Thus, other factors related to increased obesity in this population (e.g., poor diet, physical activity) may potentiate accumulation of fat cells and lipid content of adipocytes, resulting in greater fat mass in Hispanic children. Alternatively, it is possible that the association of MCPP with obesity in Aim 2 is more highly confounded by diet or other unmeasured factors within the Hispanic population.

We found evidence that Σ DEHP was associated with lower percent fat mass in Specific Aim 1, but it was not associated with body size in Specific Aim 2. It is possible that associations of Σ DEHP with body fatness are not detectable using anthropometric measurements that do not distinguish between fat and lean mass. An inverse association between DEHP and obesity has been hypothesized since DEHP is a PPAR alpha agonist and DEHP exposures cause PPAR

alpha-mediated lipid mobilization, fatty acid oxidation, and adipose tissue atrophy in animal studies (Grun and Blumberg 2009). In contrast, DEHP metabolites are also known to affect PPAR gamma, which would be expected to result in more adipocytes and increased fat mass (Grun and Blumberg 2009). Interpretation of animal studies is complicated as they have reported differences in direction of associations by animal and dose, which may reflect the fact that DEHP influences many biological processes that are related to both increased and decreased fat accumulation.

Notably, associations of MCPP with body size were positive and associations of Σ DEHP with body size were negative for all outcomes in both Aims. Because the sources of exposure to these high molecular weight phthalate metabolites are similar, i.e., food packaging and plastics, one might expect that any residual confounding would be in the same direction for both phthalates. Since food sources of these phthalates include dairy, protein, and other high fat foods, it is more plausible that confounding would be positive. Thus, if there is a true negative association of DEHP with body size, it is likely that positive confounding by diet attenuated estimates up and toward the null.

In Specific Aim 2, MEP and MBzP were associated with reduced body size among girls and non-Hispanic black children, respectively. These findings suggest that MEP and MBzP may have effects on body size, though not through an obesogenic mechanism. These metabolites may interfere with thyroid or growth hormone regulation to disrupt typical physical development. Though cross-sectional studies do not provide causal evidence, studies assessing associations between urinary phthalate metabolites and thyroid hormones have generally reported inverse associations (Boas et al. 2010; Huang et al. 2007; Meeker et al. 2007; Meeker and Ferguson 2011), consistent with a potential negative effect of MEP and MBzP on body size mediated by their inhibitory effects on thyroid function. While MEP is not thought to be anti-androgenic, we found sexually dimorphic effects in Aim 2 such that MEP was associated with reduced body size only among girls. This finding could be consistent with thyroid effects as thyroid dysfunction is

more common among women, potentially due to estrogen-mediated differences in oxidative stress (Fortunato et al. 2014).

Dibutyl phthalates have been reported in animal studies to be anti-androgenic and affect PPARs and thyroid function. However, associations of the DBP metabolites MnBP and MiBP with body size were null in both Aims. The primary routes of exposure for MnBP and MiBP are dermal application of personal care products (e.g., nail polish, cosmetics) whereas MEP exposure is primarily through inhalation of fragrances, and MCPP and DEHP exposures occur through ingestion of contaminated food. Thus, the lack of associations for DBP metabolites, which have been hypothesized to be obesogenic based on toxicological evidence, may be due to lower bioavailability of metabolite exposures via dermal absorption as compared to inhalation or ingestion.

In addition to our primary study findings regarding the relationship between phthalate exposures and body size, both Aims explored two important potential biases: 1) confounding by correlated metabolites, and 2) bias from potentially nonignorable missing outcome data. While most of our associations were null, we did not observe substantial differences in effect estimates when comparing single and multiple metabolite models or models that assumed outcomes were MAR or MNAR. While these issues should be explored in further studies, particularly where stronger associations are observed, our findings provide some evidence that correlated metabolites and missing outcome data may not result in substantial bias of associations of prenatal phthalate exposures and childhood health effects. Nevertheless, our finding that loss to follow-up may indeed depend on the child's unobserved body size is an important consideration for future studies. Our estimates were not biased, likely because phthalate exposures were not associated with loss to follow-up (i.e., there was no backdoor path from exposure to outcome through loss to follow-up). Studies of exposures that are more strongly related to subject retention may be more susceptible to selection bias through a nonignorable missing outcome mechanism.

B. Strengths and Limitations

Strengths

The current study is innovative in that it is the first prospective study of prenatal phthalate exposures and childhood body size. Previous cross-sectional analyses cannot disentangle the temporal ordering of exposure and outcome and are particularly susceptible to confounding by sources of phthalate exposure that are also related to body size. Furthermore, this study examines associations with exposures during the prenatal period, a hypothesized critical window for an effect of phthalates on growth and obesity (Dietz 1994).

In addition, the multiethnic composition of the study population is a study strength. In particular, the MSSM and CCEH cohorts include a high proportion of participants belonging to racial and ethnic groups at increased risk of childhood obesity. In the 2007 NHANES survey, overweight and obesity prevalence was higher among both non-Hispanic black (35.9%, 20.0%) and Hispanic (38.2%, 20.9%) children aged 2-19 years compared to non-Hispanic white children (29.3%, 15.3%) (Ogden et al. 2010). Thus, our findings generalize to children at highest risk of obesity.

The analysis of BIA measures of body composition in Aim 1 is also a strength of our first Aim. Because phthalates are hypothesized to affect adipocyte number and differentiation, percent body fat is a particularly relevant and sensitive endpoint for evaluating whether phthalates are environmental obesogens.

Aim 2 assessed associations in three independent cohorts with notable variation in population characteristics, supporting the robustness of our findings. This pooled analysis utilized data from the only existing US cohorts with measured prenatal phthalate exposures and longitudinal follow-up of growth in both girls and boys. Although each of the three cohorts collected data on the exposures and outcomes of interest, power to detect an association within any individual study is limited. Pooling the cohorts thus provided sufficient sample size to more

precisely estimate associations of prenatal phthalate exposures on body size, while controlling for a rich set of potential confounding variables and assessing heterogeneity of associations by factors such as child's sex and race/ethnicity.

We employed a flexible Bayesian modeling approach that accommodated methods to multiply impute missing at random covariate data and control for confounding among correlated metabolites. Previous studies typically conduct complete case analyses and assess phthalate metabolites one at a time. Accounting for these potential biases increases the robustness of our findings. Furthermore, we were able to compare results of analyses using single and multiple metabolite models and found that co-adjustment did not substantially alter our findings.

Lack of subject retention is a common problem in longitudinal cohort studies. Loss to follow-up may result in selection bias if clinic non-attendance is missing not at random. While previous studies have ignored participant drop out and conducted analyses only among subjects who returned for clinic visits, this study explored this potential bias using selection models for data missing not at random.

Limitations

As noted previously, lack of information on dietary sources of high molecular weight phthalate exposures may have resulted in confounding of associations for MCPP and Σ DEHP. Maternal consumption of meat, dairy, and other fatty foods may result in higher exposures to high molecular weight phthalates (Serrano et al. 2014). If the mother's diet is similar to her child's, then a high fat diet would also be expected to increase her child's risk of overweight or obesity. Although we adjusted for maternal pre-pregnancy BMI and gestational weight gain to control for this pathway, there may be residual confounding, most likely up and away from the null. If present, this confounding pathway might explain the association we observed in Aim 2 of MCPP with overweight/obese status in children. For Σ DEHP, associations with body size outcomes were negative and this bias would have resulted in an attenuation of effect estimates.

Similarly, we lacked information on postnatal phthalate exposures and pre- and post-natal exposures to other potentially obesogenic chemicals (e.g., persistent organic pollutants, perfluoroalkyl compounds, bisphenol A). Although childhood phthalate exposures during childhood occur temporally after pregnancy and thus cannot affect prenatal exposures, there may be unblocked backdoor paths through unmeasured predictors of both pre- and post-natal phthalate levels. In addition, there may be unmeasured confounding by other chemicals if they affect childhood obesity and are correlated with phthalate exposures.

Confounding by diet, environmental obesogens, and other factors may operate differently within different population subgroups. Thus, residual confounding of associations may contribute to differences in associations by race/ethnicity or cohort. For example, Hispanic children had higher BMIs on average than non-Hispanic children. Stronger associations of confounders with phthalate exposure and obesity among Hispanics could explain the larger magnitude of association we observed between MCPP and overweight/obesity among Hispanic children compared to non-Hispanic children. We adjusted for a number of demographic, socioeconomic, and anthropometric variables in an effort to minimize this potential bias. However, there is little information on differences in phthalate exposure sources between racial and ethnic groups, limiting our ability to characterize all important predictors of both phthalate exposure and obesity that may vary by race/ethnicity.

We measured phthalate metabolite concentrations in a single spot urine sample, which may not represent exposures throughout pregnancy since phthalates are quickly eliminated from the body (<24 hours) (Koch et al. 2005) and exposure to phthalate sources is episodic. However, many sources of phthalate exposure are frequent and may result in steady-state levels (Calafat and McKee 2006; Hauser et al. 2004). Urinary concentrations of phthalate metabolites in a single urine sample may be moderately predictive of DBP, MEP, and BzBP exposures during pregnancy, with greater variability for DEHP (Adibi et al. 2008; Braun et al. 2012; Ferguson et al. 2014a). Further, the probability of correctly classifying “high exposure”

over a 6-12 week period using a single urine sample was reported in two studies to range between 0.50 and 0.74, depending on the metabolite (Adibi et al. 2008; Hauser et al. 2004). Studies with repeat urine samples throughout pregnancy would allow for a more robust characterization of phthalate exposures and provide insight into whether the third trimester is the relevant time period for an effect. For example, the association of MCPP with overweight and obesity was null in the HOME Study. If the third trimester is a critical window for adverse effects on adipogenesis (Dietz 1994), this null association may have been due to the earlier age at urine collection in this cohort than in MSSM or CCEH.

In addition to differences in gestational age at urine collection, the study populations of the three cohorts differed by design in terms of race/ethnicity, parity, and maternal smoking during pregnancy. These differences and other, unmeasured factors complicate the interpretation of findings that differed by cohort in our second Aim. However, analyzing these data using the same methods and controlling for the same confounders helps to rule out many potential differences that would have existed had these results been published in separate studies using different methods and yields a narrower range of plausible explanations for differences.

For Specific Aim 2, adiposity is estimated using weight and BMI. Although these anthropometric measures do not directly assess adiposity, BMI is a moderately sensitive and highly specific indicator of childhood adiposity and adolescents with a high BMI are at greater risk for health risks in adulthood (Freedman and Sherry 2009). Anthropometry during childhood may also be susceptible to measurement error, particularly at young ages when children are experiencing periods of rapid growth and plateaus (e.g., adiposity rebound). Measurement error was minimized in each cohort by using trained staff, standardized protocols, and repeat measurements. However, we did not measure body size outcomes with great enough frequency to assess growth trajectories. Thus, children may have been measured just before or after a growth spurt, leading to noise in our outcome measurements.

Finally, the current study examined body size outcomes in children during a relatively narrow age range. We restricted our analysis to early childhood because phthalate exposures have been associated with delayed puberty in boys and girls (Ferguson et al. 2014b; Wolff et al. 2014), and pubertal development is also strongly tied to BMI. Similar to our finding that MEP concentrations were associated with decreased BMI z-scores among girls, Harley et al. reported an inverse association of prenatal urinary BPA concentrations with BMI and body fat among girls only, and this association was stronger among girls who had not yet reached puberty (Harley et al. 2013). Therefore, evaluating associations among older children is warranted to determine whether observed associations persist with age and to evaluate the timing of additional potential obesogenic effects with respect to puberty or other sensitive periods in development.

C. Public Health Significance

If causal, the observed association of prenatal MCPPE exposure with overweight and obesity during early childhood may have substantial public health impact since nearly 40% of US children are overweight or obese (Ogden et al. 2010). We observed over a doubling of the odds of overweight/obese status for each SD increase in natural log MCPPE concentrations (with approximately a 6 SD range in MCPPE exposure). If further research supports an association of MCPPE with childhood obesity, government regulation of its parent phthalate ester, DnOP, may help to reduce the burden of childhood obesity and does not require individual behavior change (i.e., diet and exercise) that is difficult to implement at a population level. Since the costs associated with childhood obesity are great, even a small shift in the BMI distribution could have an important impact on public health.

While our primary hypotheses posited that early life phthalate exposures are related to increased fat accumulation in children, we observed negative associations of prenatal

exposures to DEHP, DEP, and BBzP with body size. These negative associations suggest that certain phthalates may affect physical development through non-obesogenic mechanisms, possibly related to thyroid or growth hormone regulation. Phthalates have been reported to adversely affect neurodevelopment, which is also critically related to thyroid functioning. Thus, these studies contribute to a growing body of evidence suggesting that phthalate exposures during gestation may indeed interfere with normal development of the fetus.

Currently, regulations governing phthalates are discrepant across and within nations due to a lack of high quality data quantifying risks. Given the proliferation of cross-sectional studies investigating hypothesized obesogens, which are particularly prone to confounding bias, the studies conducted as part of this dissertation provide much needed prospective evidence evaluating potential risks of prenatal phthalate exposures with respect to childhood body size. While this dissertation does not indicate a strong, definitive causal association, we did observe relations of prenatal exposures to certain phthalates with increased or decreased body size in children. Given that gestation is a sensitive period in development, the precautionary principal suggests that it is prudent for pregnant women to limit their phthalate exposures when possible. However, current regulatory requirements in the US do not stipulate that phthalate-containing products be labeled as such, limiting a woman's ability to reduce her exposures.

D. Directions for Future Research

There is little toxicological evidence examining whether early life exposure to DnOP or its metabolite, MCPP, alter biologic processes related to development of obesity. Animal studies evaluating the effects of DnOP on hypothesized pathways such as PPARs alpha and gamma, thyroid and growth hormones, or androgen levels would aid in assessing the plausibility of our observation of increased overweight and obesity among prenatally-exposed children.

Given that the MSSM, CCEH, and HOME studies constitute the only existing US cohorts available for evaluating associations of prenatal environmental chemicals with childhood body

size, future analyses of these cohorts could 1) integrate measures of childhood phthalate exposures, 2) examine prenatal and childhood co-exposures to other potential obesogenic chemicals, and 3) continue to follow children through adolescence. Examining associations of phthalates measured during childhood would be beneficial to determine whether pregnancy is the relevant time period for phthalate effects and assess whether ongoing phthalate exposures alter or potentiate associations. Development of methods to assess joint effects of phthalate exposures would aid in the characterization of how phthalates may interact with each other (and with other environmental chemicals) to produce changes in growth and physical development. Finally, examining associations at older ages may provide insight as to whether the effects of prenatal phthalate exposures manifest at different developmental stages, especially given that phthalate exposures have been associated with delayed puberty.

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